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(57) Abstract: Fungi and bacteria can be detected and quantified by using a nucleotide sequence taught here that are specific to the particular species of fungi and species of bacteria. Use of the sequences can be made with fluorescent labeled probes.

## METHOD OF IDENTIFYING AND QUANTIFYING SPECIFIC FUNGI AND BACTERIA

### Cross-reference to Related Applications

The present application is a continuation in part of Serial No. 09/290,990, filed April 14, 1999, which claims priority from provisional application Serial No. 60/061,773, filed April 15, 1998, the entire contents of both of which are hereby incorporated by reference.

### Field of the Invention

The present invention relates to a method of identifying and quantifying specific fungi and bacteria using specific DNA sequences, as described and taught herein. These sequences can be used with real time detection of PCR products with a flucrogenic probe system or other molecular probes like hybridization.

### Background of the Invention

Fungi and bacteria are the source of or contribute to many health problems including infections, gastroenteritis, ulcers, asthma, allergies and sinusitis. The rapid identification of the microorganisms is critical for diagnosis and treatment. In addition, detecting and/or quantifying these microorganisms in the environment may help to prevent adverse health effects.

### Limitations of Current Technology

In order to determine the risk fungi and bacteria pose to human health it is necessary to know what fungi and bacteria are present and in what numbers. Fungi and bacteria can be ingested, inhaled, or might enter the body through

abrasions or punctures. It is important to identify these microorganisms as specifically and as rapidly as possible. Some species of a particular genus are harmless whereas others of the same genus may cause significant health effects. So without knowing precisely what microorganisms are present and in what numbers, it is impossible to evaluate the potential for negative health effects or the establishment of a risk assessment.

In the past, the detection and quantitative measurement of fungi and bacteria in samples has been performed either by direct microscopic examination of the collected cells or by growing cells on a suitable medium and identification and enumeration of the resultant colonies. The first method is highly labor intensive and is subject to potential errors in the recognition and positive identification. The second method is both time consuming and subject to significant quantitative inaccuracy. Both methods require extensive experience on the part of the analyst.

Some molecular approaches, such as the conventional polymerase chain reaction (PCR) procedure, are subject to inaccuracies due to the difficulty of quantifying the product. This procedure is also relatively slow and requires expertise in molecular biology.

Summary of the Invention

It is an object of the present invention to provide a simple, reliable method for detecting and quantifying some fungi and bacteria by using the nucleotide sequences specific to each species or group of species of fungi and bacteria, as described herein.

According to the present invention, fungi and bacteria can be identified and quantified by using a nucleotide sequence specific to the particular species or, in the case of some fungi, group of species. Many methods including using real time, probe-based detection of polymerase chain reaction (PCR) products (e.g. TaqMan™ system) or other methods of detection and quantification including hybridization or conventional PCR could be used with these sequences.

#### THEORY

Each microorganism is unique because of the sequence of some of the nucleotides in its DNA. However, there are many sequences which are common to more than one organism. There is thus a hierarchy or classification into which all microorganisms can be arranged. The "species" are typically the finest level of distinction that is recognized for separation of different members of a given genus. In the past, species were separated on the basis of morphological or biochemical differences. In order to identify or separate different species on the basis of its DNA sequence, one finds sequences that are unique to a given species but at the same

time common to all isolates of a given species.

For this invention the internal transcribed spacer (ITS) regions of nuclear ribosomal DNA (rDNA) of the different fungi were used. For the bacteria, the sequences of unique enzymes were chosen.

To apply this invention, a number of possible detection methods are possible. For example, the TaqMan™, 3'-5' exonuclease assay signals the formation of PCR amplicons by a process involving the nucleolytic degradation of a double-labeled fluorogenic probe that hybridizes to the target template at a site between the two primer recognition sequences (cf. U.S. Patent 5,876,930). The model 7700 automates the detection and quantitative measurement of these signals, which are stoichiometrically related to the quantities of amplicons produced, during each cycle of amplification. In addition to providing substantial reductions in the time and labor requirements for PCR analyses, this technology permits simplified and potentially highly accurate quantification of target sequences in the reactions.

There are additional systems and other molecular approaches that operate upon essentially the same principal. What is common to all of these technologies is the need for the identification of specific sequences that are unique to the targeted organism but common to all members of the

of the sequences in the identification and quantification of specific fungi and bacteria.

#### Brief Description of the Drawings

Figure 1 illustrates the sensitivity of the assay of the present invention.

Figure 2 shows the actual vs. the expected amounts of conidia detected.

Figure 3 shows TaqMan Threshold Responses from ten-fold dilutions of a single DNA extract.

Figure 4 shows *H. pylori* counts per assay plotted against cycle threshold values.

#### Detailed Description of the Invention

##### DNA extraction

Genomic DNA is extracted using standard methods, e.g., the glass bead milling and glass milk adsorption method or any similar procedure of extracting genomic DNA.

Reactions are prepared in 0.5 ml thin-walled, optical grade PCR tubes (PE Applied Biosystems, Foster City CA) by addition of the following components: 12.5  $\mu$ l of TaqMan Universal Master Mix (a 2X-concentrated, proprietary mixture of AmpliTaq Gold™ DNA polymerase, AmpErase® UNG, dNTPs with UTP, passive reference dye and optimized buffer components, PE Applied Biosystems, Foster City CA); 2.5  $\mu$ l of a mixture of forward and reverse primers (10 nM each); 2.5  $\mu$ l of 400 nM TaqMan probe; 1.5  $\mu$ l of 2 mg/ml bovine serum albumin, fraction V (Sigma Chemical, St. Louis, MO) and 5  $\mu$ l of DNA template.

For each targeted fungus or bacterium, the appropriate forward primer, reverse primer and probe (Table 1) are to be obtained. The probe is labeled with an appropriate set of dyes or other markers for the particular system of measurement being used.

For each target species or group of species, a calibrator sample with a known number of conidia is used as a standard. To ensure that the sample matrix does not affect the PCR reaction and, thus the quantitative results, an internal standard is used. Addition of these conidia or cells to both the test and calibrator samples normalize the target species or group for potential sample to sample variability in DNA extraction efficiencies.

Table 1. List of Fungal Primers and Probes

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*Absidia coerulea/glauca*

Forward Primer NS92F: 5'-CACCGCCCGTCGCTAC (SEQ ID NO:1)

Reverse Primer AcoerR1: 5'-TCTAGTTTGCCATAGTTCTCTTCCAG (SEQ ID NO:2)

Probe McoP1: 5'-CCGATTGCATGGTTATAGTGAGCATATGCCATC (SEQ ID NO:3)

*Absidia corymbifera*

Forward Primer NS92F: 5'-CACCGCCCGTCGCTAC (SEQ ID NO:4)

*Acremonium strictum*

Forward Primer      AstroF1: 5'-CAACCCATTGTGAACTTACCAAAC (SEQ ID NO:7)  
Reverse Primer      AstroR1: 5'-CGCCCTCAGAGAAATACGATT (SEQ ID NO:8)  
Probe                AstroP1: 5'-TCAGCGCGGGTGGCCTC (SEQ ID NO:9)

*Alternaria alternata*

Forward Primer AaltrF1: 5'-GGCGGGCTGGAACCTC (SEQ ID NO:10)  
Reverse Primer AltrR1-1: 5'-GCAATTACAAAAGGTTTATGTTTGTCGTA (SEQ ID NO:11)  
Reverse Primer AaltrR1-2: 5'-TGCAATTACTAAAGGTTTATGTTTGTCGTA (SEQ ID NO:12)  
Probe                AaltrP1: 5'-TTACAGCCTTGCTGAATTATTCACCCTTGTCCTT (SEQ ID NO:13)

*Apcphysomyces elegans* and *Saksenea vasiformis*

Forward Primer      NS92F: 5'-CACCGCCCGTCGCTAC (SEQ ID NO:14)  
Reverse Primer      AelegR1: 5'-GACTCGAATGAGTTCTCGCTTC (SEQ ID NO:15)  
Probe                AelegP1: 5'-TGGCCAGAGACCAGAATATGGGATTGC (SEQ ID NO:16)

*Aspergillus flavus/oryzae*

Forward Primer AflavF1: 5'-CGAGTGTAGGTTCTAGCGA (SEQ ID NO:17)  
Reverse Primer AflavR1: 5'-CGTTCATCAGAAAT (SEQ ID NO:18)  
Probe                AflavP1: 5'-TCCACCCCGTGTCTTACTGTACCTTAGTTGCT (SEQ ID NO:19)



*Aspergillus fumigatus, Neosartorya fischeri*

Forward Primer AfumiF1: 5'-GCCCCGCGTTTCGAC (SEQ ID NO:20)  
Reverse Primer AfumiR1: 5'-CCGTTGTTGAAAGTTTAACTGATTAC  
(SEQ ID NO:21)  
Probe AfumiP1: 5'-CCCGCCGAAGACCCCAACATG (SEQ ID  
NO:22)

*Aspergillus niger/foetidus/phoenicus*

Forward Primer AnigrF1: 5'-GCCGGAGACCCCAACAC-3' (SEQ ID NO:23)  
Reverse Primer AnigrR1: 5'-TGTTGAAAGTTTAACTGATTGCATT-3' (SEQ  
ID NO:24)  
Probe AnigrP1: 5'-AATCAACTCAGACTGCACGCTTTCAGACAG (SEQ  
ID NO:25)

*Aspergillus nomius*

Forward Primer AflavF1: 5'-CGAGTGTAGGGTTCCTAGCGA-3' (SEQ ID  
NO:26)  
Reverse Primer AnomiR1: 5'-CCGGCGGCCTTGC-3' (SEQ ID NO:27)  
Probe AflavP1: 5'-TCCCACCCGTGTTTACTGTACCTTAGTTGCT  
(SEQ ID NO:28)

*Aspergillus ochraceus/ostianus/auricomus*

Forward Primer AochrF1: 5'-AATTTCCATCCGTGTATACC-3' (SEQ  
ID NO:29)  
Reverse Primer AochrR1: 5'-CCGGCGAGCGCTGTG-3' (SEQ ID  
NO:30)  
Probe AochrP1: 5'-ACCTTGTTCCTTCGGCGAGCCC (SEQ ID  
NO:31)

Reverse Primer Aparar3: 5'-GCCCCGGGGCTGACG (SEQ ID NO:33)

Probe AflavP1: 5'-TCCCCACCCCTCTTTACTGTACCTTAGTTGCT  
(SEQ ID NO:34)

*Aspergillus restrictus/caesillus/conicus*

Forward Primer ArestF2: 5'-CGGSCCCGGCCTTCAT-3' (SEQ ID  
NO:35)

Reverse Primer ArestR1: 5'-GTTGTTGAAAGTTTTTAACGATTTTTTCT  
(SEQ ID NO:36)

Probe ArestP1: 5'-CCCGCCGGGAGACTCCAACATTG (SEQ ID  
NO:37)

*Aspergillus sydowii*

Forward Primer AsydcF1: 5'-CAACCTCCCACCCGTGAA (SEQ ID  
NO:38)

Reverse Primer versR1: 5'-CCATTGTTGAAAGTTTTGACTGATTTTA  
(SEQ ID NO:39)

Probe versP1: 5'-AGACTGCATCACTCTCAGGCATGAAGTTCAG  
(SEQ ID NO:40)

*Aspergillus tamaraii*

Forward Primer AflavF1: 5'-CGAGTGTAGGGTTCCCTAGCGA (SEQ ID  
NO:41)

Reverse Primer AtamaR1: 5'-CCCGGGCGGCCTTAA (SEQ ID NO:42)

Probe AflavP1: 5'-TCCCCACCCCTCTTTACTGTACCTTAGTTGCT  
(SEQ ID NO:43)

*Aspergillus terreus*

Forward Primer AterrF1: 5'-TTACCGAGTGCGGGTCTTTA (SEQ ID  
NO:44)

Reverse Primer AterrR1: 5'-TGGGGGTTTACGAT (SEQ ID NO:45)

Probe AterrP1: 5'-AACCTCCCACCCGTGACTATTGTACCTTG  
(SEQ ID NO:46)

*Aspergillus ustus*

*Forward Primer* AustsF1: 5'-GATCATTACCGAGTGCAGGTCT (SEQ ID NO:47)

*Reverse Primer* AustsR1: 5'-GCGGAAGCAACGTTGGTC (SEQ ID NO:48)

*Probe* AustsP1: 5'-CCCCCGGGCAGGCCTAACC (SEQ ID NO:49)

*Aspergillus versicolor*

*Forward Primer* IversF2: 5' CCGCGGGGAGCCCT (SEQ ID NO:50)

*Reverse Primer* versR1: 5'-CCATTGTTGAAAGTTTGGACTGATTTTA (SEQ ID NO:51)

*Probe* versP1: 5'-AGACTGCATCACTCTCAGGCATGAAGTTCAG (SEQ ID NO:52)

*Chaetomium globosum*

*Forward Primer* CglobF1: 5'-CCGCAGGCCCTGAAAAG (SEQ ID NO:53)

*Reverse Primer* CglobR1: 5'-CGCGGCGCGACCA (SEQ ID NO:54)

*Probe* CglobP1: 5'-AGATGTATGCTACTACGCTCGGTGCGACAG (SEQ ID NO:55)

*Cladosporium cladosporioides*Type 1

*Forward Primer* Clad1F1: 5'-CATTACAAGTGACCCCGGTCTAAC (SEQ ID NO:56)

Type 2

*Forward Primer* Colad2F1: 5'-TACAAGTGACCCCGGCTAAG (SEQ ID NO:59)  
*Reverse Primer* ColadR1: 5'-CCCGGAGGCAACAGAG (SEQ ID NO:60)  
*Probe* ColadP1: 5'-CCGGGATGTTTCATAACCCCTTTGTTGTCC (SEQ ID NO:61)

*Cladosporium herbarum*

*Forward Primer* CherbF1: 5'-AAGAACGCCCGGGCTT (SEQ ID NO:62)  
*Reverse Primer* CherbR1: 5'-CGCAAGAGTTTGAAGTGTCCAC (SEQ ID NO:63)  
*Probe* CherbP1: 5'-CTGGTTATTCATAACCCCTTTGTTGTCCGACTCTG (SEQ ID NO:64)

*Cladosporium sphaerospermum*

*Forward Primer* CsphaF1: 5'-ACCGGCTGGGTCTTTCTG (SEQ ID NO:65)  
*Reverse Primer* CsphaR1: 5'-GGGGTTGTTTTACGGCGTG (SEQ ID NO:66)  
*Probe* CsphaP1: 5'-CCCGCGGCACCCTTTAGCGA (SEQ ID NO:67)

*Conidiobolus coronatus/incongruus*

*Forward Primer* NS92F: 5'-CACCGCCCGTGGCTAC (SEQ ID NO:68)  
*Reverse Primer* ConiR1: 5'-TGACCAAGTTTGACCAATTTCTCTA (SEQ ID NO:69)  
*Probe* ConiP1: 5'-ATGGTTTATTCAGGTTCTGATGTTTGAAGTCT (SEQ ID NO:70)

*Cunninghamella elegans*

Forward Primer NS92F: 5'-CACCGCCCCGTCGCTAC (SEQ ID NO:71)  
Reverse Primer CunR1: 5'-AATCTAGTTTGCCATAGTTCTCTCTCA (SEQ ID NO:72)  
Probe CunP1: 5'-TGAATGGTCATAGTGAGCATGTGGGATCTTT (SEQ ID NO:73)

*Emericella nidulans/rugulosa/quadrilineata*

Forward Primer AversF1: 5'-CAACCTCCCACCCGTGAC (SEQ ID NO:74)  
Reverse Primer AniduR1: 5'-CATTGTTGAAAGTTTGAAGTATTGT (SEQ ID NO:75)  
Probe versP1: 5'-AGACTGCATCACTCTCAGGCATGAAGTTCAG (SEQ ID NO:76)

*Eurotium amstelodami/chevalieri/herbariorum/rubrum/repens*

Forward Primer EamstF1: 5'-GTGGGGGCACCATGTCT (SEQ ID NO:77)  
Reverse Primer EamstR1: 5'-CTGGTTAAAAAGATTGGTTGCGA (SEQ ID NO:78)  
Probe EamstP1: 5'-CAGCTGGACCTACGGGAGCGGG (SEQ ID NO:79)

*Epicoccum nigrum*

Forward Primer EnigrF1: 5'-TTGTABACTTCGGTCTCTACCTCTT (SEQ ID NO:80)  
Reverse Primer EnigrR1: 5'-TGCAACTGCATAGGGTTTGAT (SEQ ID NO:81)  
Probe EnigrP1: 5'-CATGTCTTTTGAGTACCTTCGTTTCTCTCGGC (SEQ ID NO:82)

*Reverse Primer*      GecR1: 5'-TTGATTGGAAATTTTAGAAGAGCAAA (SEQ ID NO:84)

*Probe*                GecP1: 5'-CAATTCCAGAGAGAGAAACAACGCTCAAAACAAG (SEQ ID NO:85)

*Geotrichum candidum*

*Forward Primer*      NS91F: 5'-CACCGCCCGTCGCTAC (SEQ ID NO:86)

*Reverse Primer*      GcandR1: 5'-AGAAAAGTTGCCCTCTCCAGTT (SEQ ID NO:87)

*Probe*                GecP2: 5'-TCAATCCGGAAGCCTCACTAAGCCATT (SEQ ID NO:88)

*Geotrichum klebahnii*

*Forward Primer*      NS91F: 5'-CACCGCCCGTCGCTAC (SEQ ID NO:89)

*Reverse Primer*      GklebR1: 5'-AAAAGTCGCCCTCTCCTGC (SEQ ID NO:90)

*Probe*                GecP2: 5'-TCAATCCGGAAGCCTCACTAAGCCATT (SEQ ID NO:91)

*Memnoniella echinata*

*Forward Primer*      StacF4      5'-TCCCAAACCCCTTATGTGAACC (SEQ ID NO:92)

*Reverse Primer*      MemR1: 5'-TGTTTATACCACTCAGACGATACTCAAGT (SEQ ID NO:93)

*Probe*                MemP1: 5'-CTCGGCCCCGGAGTCAGGC (SEQ ID NO:94)

*Mortierella polycephala/wolfii*

*Forward Primer*      NS91F: 5'-CACCGCCCGTCGCTAC (SEQ ID NO:95)

*Reverse Primer*      MortR1: 5'-TGACCAAGTTGGATTAAGTTTTCAG (SEQ ID NO:96)

*Probe*                MortP1: 5'-CTTAGTGAGGCTTTCGGATTGGATCTAGGCA (SEQ ID NO:97)

Reverse Primer MmuceF1: 5'-CTAAATAATCTAGTTTGCCATAGTTTTCG (SEQ  
ID NO:99)

Probe MucP1: 5'-CCGATTGAATGGTTATAGTGAGCATATGGGATC  
(SEQ ID NO:100)

amphibiorum/circinelloides/heimalis/indicus/mucedo/racemosus/  
ramosissimus and Rhizopus  
azygosporus/homothalicus/microsporus/oligosporus/oryzae

Reverse Primer MucR1-1: 5'-CCTAGTTTGCCATAGTTCTCAGCAG (SEQ ID NO:102)

Probe MucP1: 5'-CCGATTGAATGGTTATAGTGAGCATATGGGATC  
(SEQ ID NO:103)

Forward Primer MyroF1: 5'-AGTTTACAAACTCCCAAACCCTTT (SEQ ID NC:104)

Reverse Primer      MyroR1: 5'-GTGTCCTCAGAGGAGAAAACCA (SEQ ID NO:105)

Probe K16081: 5'-CGACTGGTTCGGGSCC (SEQ ID NO:116)

Forward Primer      PlilaF1: 5'-CCCACTGTGAACCTTACCTCAG (SEQ ID NO:107)

*Paecilomyces variotii*

Forward Primer FvariF1: 5'-CCCGCCGTGGTTCAC (SEQ ID NO:110)

Forward Primer FvariF2: 5'-CGAAGACCCCTGGAACG (SEQ ID NO:111)

Reverse Primer FvariR1: 5'-GTTGTTGAAAGTTTTAATTGATTGATTGT (SEQ ID NO:112)

Probe FvariP1: 5'-CTCAGACGGCAACCTTCCAGGCA (SEQ ID NO:113)

*Penicillium aurantiogriseum/polonicum/viridicatum/freii/verrucosum\*/hirsutum*

Forward Primer PauraF1: 5'-CGGGCCCGCCTTTAC (SEQ ID NO:114)

Reverse Primer PauraR1-1: 5'-GAAAGTTTTAAATAATTTATATTTTCACTCAGAGTT (SEQ ID NO:115)

Probe PenP2: 5'-CGCGCCCGCCGAAGACA (SEQ ID NO:116)

*Penicillium aurantiogriseum/polonicum/viridicatum/freii*

Forward Primer PauraF2: 5'-ACCGAGTGAGGGCCCTT (SEQ ID NO:117)

Reverse Primer PauraR6: 5'-CCCGGCGGCCAGTA (SEQ ID NO:118)

Probe PenP3: 5'-TCCAACCTCCACCCGTGTTTATTT (SEQ ID NO:119)

*Penicillium brevicompactum\*/alberechii*

Forward Primer FbrevF1: 5'-CCTTGTTGCTTCGGCGA (SEQ ID NO:120)

Reverse Primer FbrevR2: 5'-TCAGACTACAATCTTCAGACAGAGTTCTAA (SEQ ID NO:121)

Probe FbrevP1: 5'-CCGCGCTTTTGGCTGCCGGG (SEQ ID NO:122)



*Penicillium*  
*chrysogenum/griseofulvum/glandicola/coprophilum/expansum*  
and *Eupenicillium crustaceum/egyptiacum*

Forward Primer PchryF1: 5'-CGGGCCCCGCCTTAAC (SEQ ID NO:123)

Reverse Primer PchryR1-1: 5'-  
GAAAGTTTTTAAATAATTTATATTTTCACTCAGAGTA (SEQ ID NO:124)

Reverse Primer PchryR2-1: 5'-  
GAAAGTTTTTAAATAATTTATATTTTCACTCAGACCA (SEQ ID NO:125)

Probe PenP1: 5'-CGCGCCCCGCCGAAGACA (SEQ ID NO:126)

*Penicillium citrinum/sartoryi/westlingi*

Forward Primer PcitrF1: 5'-CCGTGTTGCCCGAACCTA (SEQ ID NO:127)

Reverse Primer PcitrR1: 5'-TTGTTGAAAGTTTTAACTAATTTTCGTTATAG (SEQ ID NO:128)

Probe PcitrP2: 5'-CCCCTGAACGCTGTCTGAAGTTGCA (SEQ ID NO:129)

*Penicillium corylophilum*

Forward Primer PcoryF1: 5'-GTCCAACCTCCCACCCA (SEQ ID NO:130)

Reverse Primer PcoryR3-1: 5'-GCTCAGACTGCATCTTCAGACTGT (SEQ ID NO:131)

Probe PcoryP1: 5'-CTGCCCTCTGGCCCCGCG (SEQ ID NO:132)

*Penicillium sp.*

*Reverse Primer* PdecuF3: 5'-AAAAGATTGATGTGTTCGGCAG (SEQ ID NO:134)

*Probe* PdecuF2: 5'-CGCCGGCCGGACCTACAGAG (SEQ ID NO:135)

*Penicillium echinulatum/solitum/camembertii/commune/crustosum*

*Forward Primer* PchryF1: 5'-CGGGCCCGCCTTAAC (SEQ ID NO:136)

*Reverse Primer* PauraR1-1: 5'-  
GAAAGTTTTAAATAATTTATATTTTCACTCAGAGTT (SEQ ID NO:137)

*Probe* PenF2: 5'-CGCGCCCGCCGAAGACA (SEQ ID NO:138)

*Penicillium expansum/coprophilum*

*Forward Primer* PauraF2: 5'-ACCGASTGAGGGCCCTT (SEQ ID NO:139)

*Reverse Primer* PchryR6: 5'-CCCGGCGGCCAGTT (SEQ ID NO:140)

*Probe* PenP3: 5'-TCCAACTCCACCCCGTGTATTATTT (SEQ ID NO:141)

*Penicillium fellutanum/charlesii*

*Forward Primer* PfellF1: 5'-AAGCTCCACCCCGTGTATACTTA (SEQ ID NO:142)

*Reverse Primer* PfellR1: 5'-CTTATCGCTCAGACTGCAAGGTA (SEQ ID NO:143)

*Probe* PfellF1: CGGTTGCCCGCCCGGCG (SEQ ID NO:144)

*Penicillium janthinellum/raperi*

*Forward Primer* PjantF2: 5'-CCACCCCGTGTATTATCATACCTA (SEQ ID NO:145)

*Reverse Primer* PjantF2: 5'-TTGAAAGTTTAACTGATTTAGCTAATCG (SEQ ID NO:146)

*Probe* PjantF2: 5'-TGCAATCTTCAGACAGCGTTCAGGG (SEQ ID NO:147)

*Penicillium madriti/gladioli*

Forward Primer PauraF1: 5'-CGGGCCCGCCTTTAC (SEQ ID NO:148)

Reverse Primer PchryR1-1: 5'-  
GAAAGTTTAAATAATTTATATTTTCACTCAGAGTA (SEQ  
ID NO:149)

Reverse Primer PchryR2-1: 5'-  
GAAAGTTTAAATAATTTATATTTTCACTCAGACCA (SEQ  
ID NO:150)

Probe PenP2: 5'-CGCGCCCGCGGAAGACA (SEQ ID NO:151)

*Penicillium oxalicum*

Forward Primer PoxalF1: 5'-GGGCCCCGCTCAG (SEQ ID NO:152)

Reverse Primer PoxalR1: 5'-GTTGTTGAAAGTTTAACTGATTTAGTCAAGTA  
(SEQ ID NO:153)

Probe PoxalP1: 5'-ACAAGAGTTGCTTTGTGTGTCTTCGGCG (SEQ  
ID NO:154)

*Penicillium roquefortii*

Forward Primer PchryF1: 5'-CGGGCCCGCCTTAAC (SEQ ID NO:155)

Reverse Primer ProguR2: 5'-TTAAATAATTTATATTTGTTCTCAGACTGCAT  
(SEQ ID NO:156)

Probe PenP2: 5'-CGCGCCCGCGGAAGACA (SEQ ID NO:157)

*Penicillium simplicissimum/ochrochloron*

Forward Primer PsimpF1-1: 5'-AACCTCCCAACCGTGTGATT (SEQ ID  
NO:158)

Reverse Primer PsimpF2-1: 5'-GAGATCCGTTGTTGAAAGTTTATCTG (SEQ  
ID NO:159)

Penicillium simplicissimum/ochrochloron

*Penicillium simplicissimum/glaucum/thomii/pupurescens*  
and *Eu-penicillium lapidosum*

Forward Primer PspinF1: 5'-GTACCTTGTTGCTTCGGTGC (SEQ ID NO:162)

Reverse Primer PspinR1: 5'-CGTTGTTGAAAGTTTAACTTATTTAGTTTAT (SEQ ID NO:163)

Probe PspinP1: 5'-TCCGCGCGCACCGGAG (SEQ ID NO:164)

*Rhizomucor miehei/pusillus/variabilis*

Forward Primer RNS92F: 5'-CACCGCCCGTCGCTAC (SEQ ID NO:165)

Reverse Primer RmucR1: 5'-GTAGTTTGCCATAGTTCCGGTA (SEQ ID NO:166)

Probe RmucP1: 5'-TTGAATGGCTATAGTGAGCATATGGGAGGCT (SEQ ID NO:167)

*Rhizopus stolonifer*

Forward Primer RNS92F: 5'-CACCGCCCGTCGCTAC (SEQ ID NO:168)

Reverse Primer RstolR1: 5'-GCTTAGTTTGCCATAGTTCTCTAACAA (SEQ ID NO:169)

Probe RmucP1: 5'-CCGATTGAATGGTTATAGTGAGCATATGGGATC (SEQ ID NO:170)

*Scopulariopsis asperula*

Forward Primer SCbrvF1: 5'-CCCCTGCGTAGTAGATCCTACAT (SEQ ID NO:171)

Reverse Primer SCasprR1: 5'-TCGAGGTCAAACCATGAGTAA (SEQ ID NO:172)

Probe SCopP1: 5'-TCGCATCGGGTCCCGGCG (SEQ ID NO:173)

*Scopulariopsis brevicaulis/fusca*

Forward Primer SCbrvF1: 5'-CCCCTGCGTAGTAGATCCTACAT (SEQ ID NO:174)

Reverse Primer SCbrvR1: 5'-TCGAGGTCAAACCATGAAATA (SEQ ID NO:175)

Probe SCopP1: 5'-TCGCATCGGGTCCCGGCG (SEQ ID NO:176)

*Scopulariopsis brumptii*

Forward Primer SCbrmF1: 5'-CCGCTGCGTAGTAGTAAAACCA (SEQ ID NO:177)

Reverse Primer SCbrmR1: 5'-CCGAGGTCAAACATCTTTGG (SEQ ID NO:178)

Probe ScopP1: 5'-TCGCATCGGGTCCCGGCG (SEQ ID NO:179)

*Scopulariopsis chartarum*

Forward Primer SCchrF1: 5'-CCCCCTGCGTAGTAGTAAAGC (SEQ ID NO:180)

Reverse Primer SCchrR1: 5'-TCGAGGTCAAACCATCAAG (SEQ ID NO:181)

Probe ScopP1: 5'-TCGCATCGGGTCCCGGCG (SEQ ID NO:182)

*Scopulariopsis sphaerospora*

Forward Primer SCsphF1: 5'-CCCCCTGCGTAGTAGTTTACAA (SEQ ID NO:183)

Reverse Primer SCsphR1: 5'-CCGAGGTCAAACCATCAAAAG (SEQ ID NO:184)

Probe ScopP1: 5'-TCGCATCGGGTCCCGGCG (SEQ ID NO:185)

*Stachybotrys chartarum*

Forward Primer StacF4 TCCCAAGCCCTTATGTGAAC (SEQ ID NO:186)

Reverse Primer StacR5 GTTTGCCACTCAGAGAATACTGAAA (SEQ ID NO:187)

Probe StacP2 CTGCGCCCGGATCCAGGC (SEQ ID NO:188)

Reverse Primer      TasprR2-1: 5'-GGACTACAGAAAGAGATTGGTTGCTT  
(SEQ ID NO:190)

Probe              TridP1: 5'-CCAAACTGTTGCCTCGGCGGG (SEQ ID  
NO:191)

*Trichoderma asperellum/hamatum/viride\**

Forward Primer      TasprF1: 5'-CCCAAACCCAATGTGAACGT (SEQ ID  
NO:192)

Reverse Primer      TasprR1: 5'-TTTGCTCAGATCTGTAAGAAATACG (SEQ  
ID NO:193)

Probe              TridP1: 5'-CCAAACTGTTGCCTCGGCGGG (SEQ ID  
NO:194)

*Trichoderma harzianum*

Forward Primer      TharzF1: 5'-TTGCCTCGGCGGGGAT (SEQ ID  
NO:195)

Reverse Primer      TharzR1: 5'-ATTTTCGAAAAGCCTACGAGA (SEQ ID  
NO:196)

Probe              TharzP1: 5'-CTGCCCCGGGTGCCTCG (SEQ ID  
NO:197)

*Trichoderma longibrachiatum/citroviride*

Forward Primer      TlongF1: 5'-TGCCTCGGCGGGGATTC (SEQ ID  
NO:198)

Reverse Primer      TlongR1: 5'-CGAGAAAGGCTCGAGCAAAAAT (SEQ  
ID NO:199)

Probe              TlongP1: 5'-TCGCAGCCCCCGGATCCCA (SEQ ID  
NO:200)

*Trichoderma viride\*/atroviride/koningii*

Forward Primer      TviriF1: 5'-CCCAAACCCAATGTGAACCA (SEQ ID  
NO:201)

Reverse Primer      TviriR1: 5'-TCCGCGAGGGGACTACAG (SEQ ID  
NO:202)

Probe                      TridP1: 5'-CCAAACTGTTGCCTCGGCGGG (SEQ ID NO:203)

*Ulocladium atrum/chartarum*

Forward Primer UatrmF1: 5'-GCGGGCTGGCATCCTT (SEQ ID NO:204)

Reverse Primer UatrmR1: 5'-TTGTCTATGGTGGGCGAA (SEQ ID NO:205)

Probe                      UloP1: 5'-TGAATTATTCACCCGTGTCTTTTGCCTACTTCT (SEQ ID NO:206)

*Ulocladium botrytis*

Forward Primer UbotrF1: 5'-CCCCAGCAGTGGCGTT (SEQ ID NO:207)

Reverse Primer UbotrR1: 5'-CTGATTGCAATTACAAAAGGTTTATG (SEQ ID NO:208)

Probe                      UloP1: 5'-TGAATTATTCACCCGTGTCTTTTGCCTACTTCT (SEQ ID NO:209)

*Wallemia sebi*

WsebiF1: 5'-GGCTTAGTGAATCCTTCGGAG (SEQ ID NO:210)

WsebiR1: 5'-GTTTACCCAACTTTGCACTCCA (SEQ ID NO:211)

WsebiP1: 5'-TGTGCCGTGCGCGGTCAAATAG (SEQ ID NO:212)

Universal Fungal

ASSAY 1

Forward Primer              5.8F1: 5'-AACTTTCACCAACGGATCTCTTGG (SEQ ID NO:213)

Reverse Primer              5.8R1: 5'-GCGTTCAAAGACTCGATGATTCAC (SEQ ID NO:214)

Reverse Primer      ZygR1: 5'-TAATGATCCTTCGGCAGGTTTC (SEQ ID NO:217)

Probe                ZygP1: 5'-  
CCTACGGAAACCTTGTACGAJTTTTACTTCCTCTAAA  
(SEQ ID NO:218)

\* Assay does not detect all strains of the indicated species

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#### FUNGI

##### *Aspergillus auricomus*

Forward Primer      AcircF1: 5'-ATTACTGAGTGAGGGTCCCTCG

Reverse Primer      AauriR1: 5'-GGGGGCGCGGTAAAC

Probe                AcircP1: 5'-CCGGCGGAAGCAACAAGGTACG

##### *Aspergillus caespitosus*

Forward Primer      AcaesF1: 5'-CTCCCACCCGTGAATACCTT

Reverse Primer      AcaesR1: 5'-GGCTCAGACGCCAACTCTACAAT

Probe                AcaesP1: 5'-CACTGTTGCTTCGGCGAGGAGCC

##### *Aspergillus candidus*

Forward Primer      AcandF1: 5'-TTACCGAGTGAGGGTTTCTCTGA

Reverse Primer      AcandR1: 5'-ACASTGTTGTTGTTGGGTTCTT

Probe                PsimpP1: 5'-CCGGCTCAGGGCGCGCC

##### *Aspergillus cervinus*

Forward Primer      AcervF1: 5'-CCACCCGTGCTATTGTACCTTT

Reverse Primer      AcervR1-1: 5'-CAACACAGATTCGATTCGAGACTGT

Probe                AfumiP2: 5'-TTCTCGGGCGGGCGCGG

##### *Aspergillus clavatus*



Forward Primer      AclavF1: 5'-CCCGCGGTCTTCGGA  
Reverse Primer      AclavR1: 5'-CGTTGTTGAAAGTTTAACTGATTATG  
Probe                AfumiF1: 5'-CCCGCCGAAGACCCCAACATG

*Aspergillus flavipes*

Forward Primer    AflvpF1: 5'-CCACCCGTGACTACTGTACCAC  
Reverse Primer    AflvpR1: 5'-CCGGCGGCCAGCTAG  
Reverse Primer    AflvpR2: 5'-AGGCTTTCAGAAACAGTGTTCG  
Probe              AspP1: 5'-TTGCTTCGGCGGGGCC

*Aspergillus niveus*

Forward Primer    AniveF1: 5'-ACCCGTGCCTATTGTACCCT  
Reverse Primer    AniveR1: 5'-TGCAAACAATCACACTCAGACAC  
Probe              AspP1: 5'-TTGCTTCGGCGGGGCC

*Aspergillus ochraceus*

Forward Primer    AochrF1: 5'-AACCTCCCACCCGTGTATACC  
Reverse Primer    AochrR2-1: 5'-CGGCGAGCGCTGTtCC  
Probe              AochrF1: 5'-ACCTTCTTGCTTCGGCGAGCCC

*Aspergillus ostianus*

Forward Primer    AochrF1: 5'-AACCTCCCACCCGTGTATACC  
Reverse Primer    AostiR1-1: 5'-CGGCGAGCGCTGTtCF

*Aspergillus paradoxus*

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Reverse Primer    ApardR1-1: 5'-GACTGCAACTTCATACAGAGTTGGT

Probe            FenP2: 5'-CGCGCCCGCCGAAGACA

*Aspergillus penicillicides*

Forward Primer    ApeniF2: 5'-CGCCGGAGACCTCAACC

Reverse Primer    ApeniR2: 5'-TCGGTTGTTGAAAGTTTTAACGA

Probe            ApeniP2: 5'-  
TGAACACTGTCTGAAAGTTGCAGTCTGAGTATG

*Aspergillus sclerotiorum*

Forward Primer    AcircF1: 5'-ATTACTGAGTGAGGGTCCCTCG

Reverse Primer    AsclrR1: 5'-CCTAGGGAGGGGGGTTTGA

Probe            AcircF1: 5'-CCCGCCGAAGCAACAAGGTACG

*Aspergillus sydowii*

Forward Primer    AsydoF1-1: 5'-CAACCTCCCACCCGaGAA

Reverse Primer    versR1: 5'-CCATTGTTGAAAGTTTTGACTGATTTTA

Probe            versF1: 5'-AGACTGCATCACTCTCAGGCATGAAGTTCAG

*Aspergillus unguis*

Forward Primer    AunguF1: 5'-CAACCTCCCAACCTTGAATACT

Reverse Primer    AunguR1: 5'-TCACTCTCAGGCATGAAGTTCAG

Probe            AcaesP1: 5'-CACTGTTGCTTCGGCGAGGAGCC

*Aspergillus wentii*

Forward Primer    AwentF1: 5'-CATTACCGAGTGAGGACCTAACC

Reverse Primer    AwentR1: 5'-CGGCGGCGCGGAT

Probe                      AcircP1: 5'-CCCGCCGAAGCAACAAGGTACG

*Candida albicans*

Forward Primer   CalbF1: 5'-CTTGGTATTTTGCATGTTGCTCTC

Reverse Primer   CalbR1: 5'-GTCAGAGGCTATAACACACAGCAG

Probe                CalbP1: 5'-TTTACCGGGCCAGCATCGGTTT

*Candida dubliniensis*

Forward Primer   CdubF1: 5'-AGATCAGACTTGGTATTTTGCAAGTTA

Reverse Primer   CdubR1: 5'-TAGGCTGGCAGTATCGTCAGA

Probe                CdubP1: 5'-TTTACCGGGCCAGCATCGGTTT

*Candida (Pichia) guilliermondii*

Forward Primer   CguiF1: 5'-CCTTCGTGGCGGGGTG

Reverse Primer   CguiR1: 5'-GCAGGCAGCATCAACGC

Probe                CguiP1: 5'-CCGCAGCTTATCGGGCCAGC

*Candida haemulonii*

Forward Primer   ChaeF1: 5'-GGAGCGACAAACGAGCAGTC

Reverse Primer   ChaeR1: 5'-AGGAGCCAGAAAGCAAGACG

Probe                ChaeP1: 5'-ATGTAGTACAGCCCTCTGGGCTGTGCA

*Candida lusitana*      ClusF1: 5'-TGGTATGTAATGTTTCTG

Probe Cha2P1: 5'-AAGTGGGAGCTGATGTAGCAACCCCC

*Candida krusei*

Forward Primer CkruF1: 5'-CTCAGATTTGAAATCGTGCTTTG

Reverse Primer CkruR1: 5'-GGGGCTCTCACCCTCCTG

Probe CkruP1: 5'-CACGAGTTGTAGATTGCAGGTTGGAGTCTG

*Candida lipolytica*

Forward Primer ClipF1: 5'-TAGCGAGACGAGGGTTACAAATG

Reverse Primer ClipR1: 5'-CGTCGGGTGGCACTGTGGA

Probe ClipP1: 5'-CCTTCGGGCGTTCTCCCTAACC

*Candida lusitanae*

Forward Primer ClusF1: 5'-GGGCCAGCGTCAAATAAAC

Reverse Primer ClusR1: 5'-CGCAGGCCTCAAACAAACA

Probe ClusP1: 5'-AGAATGTGGCGCGTGCCTTCG

*Candida maltosa*

Forward Primer CmalF1: 5'-GGCCAGCATCACTTTGGAC

Reverse Primer CmalR1: 5'-TCTAGACTGGCAGTATCGACAGTG

Probe CmalP1: 5'-TAGGACAATTGCGGTGGAATGTGGC

*Candida parapsilosis*

Forward Primer CparF1: 5'-GATCAGACTTGCTATTTTGTATGTTACTCTC

Reverse Primer CparR1: 5'-CAGAGCCACATTTCTTTGCAC

Probe CparP1: 5'-CCTCTACAGTTTACCGGGCCAGCATCA

*Candida sojae*

Forward Primer CsojF1: 5'-CGGTTGTGTGTTATAGCCTTCGTA  
Reverse Primer CsojR1: 5'-ATCATTATGCCAACATCCTAGGTAAT  
Probe CtropP2: 5'-CGCAGTCCTCAGTCTAGGCTGGCAG

*Candida tropicalis*

Forward Primer CtropF1: 5'-GCGGTAGGAGAATTGCCTT  
Reverse Primer CtropR2: 5'-TCATTATGCCAACATCCTAGGTTTA  
Probe CtropP2: 5'-CGCAGTCCTCAGTCTAGGCTGGCAG

*Candida viswanathii*

Forward Primer CvisF1: 5'-CGGCAGGACAATCGCGT  
Reverse Primer CvisR1: 5'-TCTAGGCTGGCAGTATCCACG  
Probe CvisP1: 5'-AATGTGGCACGGCCTCGGC

*Candida zeylanoides*

Forward Primer Czey F1: 5'-GTTGTAATTTGAAGAAGSTAACCTTTGATT  
Reverse Primer Czey R1: 5'-GACTCTTCGAAAGCACTTTACATGG  
Probe Czey P1: 5'-CCTTGGAAACAGGACCTCACAGAGGGT

*Emmericella (Aspergillus) nidulans/rugulosa/quadrilineata*

Forward Primer AversF1: 5'-CAACCTCCCACCCGTGAC  
Reverse Primer AniduR1-1: 5'-CCATTGTTGAAAGTTTTACTGATaTGT

*Aspergillus nidulans*

Forward Primer GklebF1: 5'-GGGCGACTTTTTCGGC  
Reverse Primer GklebR2: 5'-TGGGACAAATTCTCCTCTAATTTATTTA  
Probe GklebP1: 5'-  
AAGCTAGTCAAACCTTGGTCATTTAGAGGAAGTAAAAGTC

*Penicillium aethiopicum*

Forward Primer PaethF1-1: 5'-CGGGGGGCTCtCGCT  
Reverse Primer PchryR1-1: 5'-  
GAAAGTTTTAAATAATTTATATTTTCACTCAGAgTA  
Probe PenP2: 5'-CGCGGCCCGCGAAGACA

*Penicillium atramentosum*

Forward Primer PgrisF1-1: 5'-ACCTGCGGAAGGATCATTtCT  
Reverse Primer PatraR1: 5'-CCCCGGCGGCCATA  
Probe PenP3: 5'-TCCAACCTCCCACCCGTGTTTATTT

*Penicillium aurantiogriseum*

Forward Primer PauraF3: 5'-CGCCGGGGGGCTTC  
Reverse Primer PauraR1-1: 5'-  
GAAAGTTTTAAATAATTTATATTTTCACTCAGAgTE  
Probe PenP2: 5'-CGCGCTCGCCGAAGACA

*Penicillium aurantiogriseum/polonicum/viridicatum/freii*

Forward Primer PexpaF1-1: 5'-TTACCGASTBAGGSCCgTT  
Reverse Primer PauraR6: 5'-CCCGCGGGCCAGTA  
Probe PenP3: 5'-TCCAACCTCCCACCCGTGTTTATTT

*Penicillium canescens*

Forward Primer      PcanEF1: 5'-TTACCGAGCGAGAATTCTCTGA  
Reverse Primer      PcanER1: 5'-AGACTGCAATTTTCATACAGAGTTCA  
Probe                PsimpP1: 5'-CCGCCTCACGGCCGCC

*Penicillium citreonigrum*

Forward Primer      PcteoF1-1: 5'-TGTTGGGCTCCGTCCTCtTC  
Reverse Primer      PcteoR1-1: 5'-CGGCCGGGCCTtCAG  
Probe                PenP7: 5'-CCGAAAGGCAGCGGCGGC

*Penicillium coprophilum*

Forward Primer      PcoprF1-1: 5'-GGGTCCAACCTCCCACtCA  
Reverse Primer      PchryR1-1: 5'-  
GAAAGTTTTAAATAATTTATATTTTCACTCAGAgTA  
Probe                PenP1: 5'-CGCCTTAACTGGCCGCCCG

*Penicillium crustosum*

Forward Primer      PcrusF1: 5'-CGCCGGGGGGGCTTA  
Reverse Primer      PauraR1-1: 5'-  
GAAAGTTTTAAATAATTTATATTTTCACTCAGAgTT  
Probe                PenP2: 5'-CGCGCCCGCCGAAGACA

*Penicillium digitatum*

Probe                PdigP1: 5'-CTGCTGCTTAAATTA

*Penicillium expansum*

(assay 1)

Forward Primer      PexpaF1-1: 5'-TTACCGAGTGAGGGCCgTT  
Reverse Primer      PexpaR2-1: 5'-GCCCCGCCGAAGctACG  
Probe                PenP3: 5'-TCCAACCTCCCACCCGTGTTTATTT

(assay 2)

Forward Primer      PexpaF2-1: 5'-TCCCACCCGTGTTTATTTACaTC  
Reverse Primer      PexpaR1: 5'-TCACTCAGACGACAATCTTCAGG  
Probe                PenF1: 5'-CGCCTTAACTGGCCGCCGG

*Penicillium freeii*

Forward Primer      PfreiF1: 5'-TCAGCCCCCGGGT  
Reverse Primer      PauraR1-1: 5'-  
                      GAAAGTTTTAAATAATTTATATTTTCACTCAGAgTT  
Probe                PenP2: 5'-CGCGCCCGCCSAAGACA

*Penicillium glandicola*

Forward Primer      PglanF1-1: 5'-CCGGGGGGCTTtCGT  
Reverse Primer      PchryR1: 5'-  
                      GAAAGTTTTAAATAATTTATATTTTCACTCAGACTA  
Probe                PenP2: 5'-CGCGCCCGCCSAAGACA

*Penicillium griseofulvum*

Forward Primer      PgrisF1-1: 5'-ACCTGCGGAAGGATCATTtCT  
Reverse Primer      PchryR6: 5'-CCCGCGGGCCAGTT  
Probe                PenP3: 5'-TCCAACCTCCCACCCGTGTTTATTT



*Penicillium hirsutum\**

Forward Primer PhirsF1-1: 5'-GCCGGGGGGGCTCAtA  
Reverse Primer FauraR1-1: 5'-  
GAAAGTTTTTAAATAATTTATATTTTCACTCAGAgTT  
Probe PenP2: 5'-CGCGCCCGCCGAAGACA

*Penicillium implicatum*

Forward Primer PimplF1: 5'-GCCGAAGACCCCCCTGT  
Reverse Primer PimplR1: 5'CSTTGTTSAAGTTTTGACTGATTGT  
Probe PimplP1: 5'-AACGCTSTCTGAAGCTTGCAGTCTGAGC

*Penicillium islandicum*

Forward Primer Pislaf1: 5'-CGAGTGCGGGTTGACA  
Reverse Primer Pislaf1: 5'-GGCAACGCGGTAAACGGTAG  
Probe Pislaf1: 5'-AGCCCCAACCTCCCACCCGTG

*Penicillium italicum*

Forward Primer PitalF1-1: 5'-CTCCACCCCGTSTTTATTTAtCA  
Reverse Primer PempaR1: 5'-TCACTCAGACGACATCTTCAGG  
Reverse Primer PempaR1-1: 5'-TCACTCAGACGACATCTTCtGG  
Probe PenF1: (+) 5'-CGCCTTAAGTGGCCGCCGG

*Penicillium melinii*

1000 1000 1000 1000 1000 1000 1000 1000 1000 1000

*Penicillium miczynskii*

Forward Primer PmiczF1-1: 5'-GTGTTTAACGAACCTTGTTGCaTT  
Reverse Primer PmiczR1-1: 5'-CTCAGACTGCATACTTCAGACaGA  
Probe PsimpF1: 5'-CCGCGCTCACGGCCGCGC

*Penicillium olsonii*

Forward Primer PolsnF1: 5'-GGCGAGCCTGCC'TTCG  
Reverse Primer PenR2: 5'-GATCCGTTGTTGAAAGTTTAAATAATTTATA  
Probe PolsnF2: 5'-TCCGCGCTCGCCGGAGAC

*Penicillium purpurogenum*

Forward Primer PpurpF1: 5'-AGGATCATTACTGASTGCGGA  
Reverse Primer PpurpR1: 5'-GCCAAAGCAACAGGGTATTTC  
Probe PpurpF1: 5'-CCCTCGCGGGTCCAACCTCC

*Penicillium raistrickii*

Forward Primer PgrisF1-1: 5'-ACCTGCGGAAGGATCATTtCT  
Reverse Primer PraisR1: 5'-CCCGGCGGCCAGAC  
Probe PenP3: 5'-TCCAACCTCCCACCCGTGTTTATTT

*Penicillium restrictum*

Forward Primer PrestF1-1: 5'-CACGGCTTGTGTGTTGGGtCT  
Reverse Primer PrestR1-1: 5'-CGGCCGGGGCCTaCAA  
Probe PenP7: 5'-CCGAAAGGCAGGGGGCGC

*Penicillium sclerotiorum*

Forward Primer PsclrF1: 5'-TTCCCCCGGGAACAGG  
Reverse Primer PsclrR1: 5'-GCCCCATACGCTCGAGGAT  
Probe PsclrP1: 5'-CCGAAAGGCAGTGGCGGCAC

*Penicillium simplicissimum/ochrochloron*

Forward Primer PsimpF2-1: 5'-CGCCGAAGACACCATTGAtCT  
Reverse Primer PsimpR4-1: 5'-CTGAATTCTGCAATTCACATaACG  
Probe PsimpP2: 5'-TGTCTGAAGATTGCAGTCTGAGCGATTAGC

*Penicillium variabile*

Forward Primer PvarbF1: 5'-GCCGGGGGGGCTTCT  
Reverse Primer PvarbR1: 5'-TCTCACTCAGACTCACTGTTCAGG  
Probe PvarbP1: 5'-AGGGTTCTAGGGTGCTTCGGCGG

*Penicillium verrucosum\**

Forward Primer PverrF2: 5'-CGGGGCCCGCCTTTG  
Reverse Primer PauraR1: 5'-  
GAAAGTTTTAAATAATTTATATTTTCACTCAGACTT  
Probe PenP3: 5'-CGCGCCCGCCGAAGAC

*Penicillium waksmanii*

Forward Primer F waksF1-1: 5'-GTGTTTAACGACCTTGTGCATC  
Reverse Primer R waksR1-1: 5'-CTTCAGACAGCGTTACAGCTAG

*Penicillium citrinum*

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Forward Primer      UatrmF2: 5'-CGGGCTGGCATCCTTC  
Reverse Primer      UatrmR2: 5'-CTGATGCAATTACAAAAGGTTTATG  
Probe                UloP1: 5'-  
                      TGAATTATTCACCCGTGTCTTTTGCSTACTTCT

*Ulocladium chartarum*

Forward Primer      UcharF1-1: 5'-AGCGGGCTGGAATCCaTT  
Reverse Primer      UcharR1-1: 5'-CTGATTGCAATTACAAAAGGTTgAAT  
Probe                UloP1: 5'-  
                      TGAATTATTCACCCGTGTCTTTTGCSTACTTCT

## Universal Fungal

Forward Primer      5.8F1-1: 5'-AACTTTCAACAACGGATCTCTTG  
Reverse Primer      5.8R1-1: 5'-CGTTCAAAGACTCGATGATTCAC  
Probe                5.8P1: 5'-CATCGATGAAGAACGCAGCGAAATGC

## BACTERIA

*Legionella maceachernii*

Forward Primer      LmaceF1: 5'-GGTGSTTTAGTAAGTTATCTGTGAAATTC  
Reverse Primer      PmaceR1: 5'-CACTACCCCTCTCCTATACTCTTAGTCCAG  
Probe                LmicdP1:  
                      5'-AGTCTTATCTGACCACCCTAGGTTGAGGCCA

*Legionella micdadei*

Forward Primer      LmicdF1: 5'-GGTGSTTTTATAAGTTATCTGTGAAATTC

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Reverse Primer PmicdR1: 5'-CACTACCCTCTCCTATACTCAAAGTCTC  
Probe LmicdF1:  
5'-AGTCTTATCTGACCACCCTAGGTTGAGCCCA

*Legionella pneumophila*

(Type1)

Forward Primer LpneuF1: 5'-CGGAATTACTGGGCGTAAAGG  
Reverse Primer PpneuR1: 5'-GAGTCAACCAGTATTATCTGACCGT  
Probe LpneuF1:  
5'-AAGCCCAGSAATTTACAGATAACTTAATCAACCA

(Type 2)

Forward Primer LpneuF2: 5'-CCCAGCTTTGTCCTCAGAC  
Reverse Primer LpneuR2: 5'-AGTCGAACGGCAGCATTG  
Probe LpneuP2: 5'-TGCTAGACAGATGGCGAGTGGCGA

*Legionella sainthelensi/cincinnatiensis*

Forward Primer LsainF1: 5'-CGTAGGAATATGCCTTGAAAGACT  
Reverse Primer PsainR1: 5'-AAGGTCCCCAGCTTTCGT  
Probe LsainF1:  
5'-AGACATATTCGGTATTATCCTTGAGTTTCCC

*Aeromonas hydrophila*

Forward Primer AhydF1: 5'-TGCCGCGTGTGTCAAGAA

*Aeromonas media/eucrenophila*

Forward Primer AmedF1: 5'-ATGCGCGCTGTGTGAAGA

Reverse Primer AmedR1: 5'-CGAGTAACGTCACAGCTGATG

Probe AmedP1: 5'-AAGCACTTTCAGCGAGGAGGAAAGGTTG

*Aeromonas schubertii*

Forward Primer AschF1: 5'-AGCGAGGAGGAAAGGTTGGT

Reverse Primer AschR1: 5'-GGAGTTAGCCGGTGCTTCTTC

Probe AschP1: 5'-TGGAGTAACGTCACAGCTGGCAGGTAT

*Aeromonas veroni*

Forward Primer AverF2: 5'-AGCGAGGAGGAAAGGTTGGTAG

Reverse Primer AverR2: 5'-CGGAGTTAGCCGGTGCTTC

Probe AverP2: 5'-TAATAACTGCCAGCTGTGGACGTTACTCGCA

*Aeromonas caviae, trota, jandaei*

Forward Primer AverF2: 5'-AGCGAGGAGGAAAGGTTGGTAG

Reverse Primer AcavR1: 5'-CGGAGTTAGCCGGTGCTTC

Probe AcavP1: 5'-TCTGCGAGTAACGTCACAGCCAGCAGATA

*Aeromonas all species*

Forward Primer AuniF1: 5'-CAGGGCTACACAGGTGCTACA

Reverse Primer AuniR1: 5'-GGGATTCGCTCAGTATCGCT

Probe AuniP1: 5'-TGGCGCGTACAGAGGGCTGCA

Table 2. List of Bacterial Primers and Probes

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*Escherichia coli*

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Forward Primer	uidAF1: 5'-GGGCAGGCCAGCGTATC (SEQ ID NO:219)
Reverse Primer	uidAR1: 5'-CCCACACTTTGCCGTAATGA (SEQ ID NO:220)
Reverse Primer	uidAR2: 5'-CGTACACTTTCCCGSCAAT (SEQ ID NO:221)
Probe	uidAF1: 5'-TGCTGCGTTTCGATCCCGTCA (SEQ ID NO:222)

*Helicobacter pylori*

Forward Primer	HpylF1: 5'-GGGTATTGAAGCGATGTTTCCT (SEQ ID NO:223)
Reverse Primer	HpylR1: 5'-GCTTTTTTGCCTTCGTTGATAGT (SEQ ID NO:224)
Probe	HpylP1: 5'-AAACTCGTAACCGTGCATACCCCTATTGAG (SEQ ID NO:225)

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One skilled in the art will appreciate that primers and/or probes can be used which are not identical to the ones described above, as long as there is substantial similarity between the sequences. Of purposes of the present invention, "substantial similarity" means that more than 90-110% of the sequence is the same as the sequences enumerated above.

Performance of Assay

Standard procedures for the operation of the model

attained with the system described herein which was

changed from 50 to 25  $\mu$ l. Thermal cycling conditions consisting of two min at 50° C, 10 min at 95° C, followed by 40 cycles of 15 sec at 95° C and 1 min at 60° C. Cycle threshold ( $C_T$ ) determinations, i.e. non-integer calculations of the number of cycles required for reporter dye fluorescence resulting from the synthesis of PCR products to become significantly higher than background fluorescence levels were automatically performed by the instrument for each reaction using default parameters. Assays for fungal target sequences and *G. candidum* (reference) sequences in the same DNA samples are performed in separate reaction tubes.

#### Quantification of fungal target

Quantification is performed by first subtracting mean reference sequence  $C_T$  values from mean target sequence  $C_T$  values for both test samples and a pre-specified calibrator sample to obtain  $\Delta C_T$  values. Calibrator sample  $\Delta C_T$  values are then subtracted from  $\Delta C_T$  values of the test samples to obtain  $\Delta\Delta C_T$  values. Assuming an amplification efficiency of one (i.e. a doubling of the target sequence for each cycle), the ratio of target sequences in the test and calibrator samples is given by  $2^{-\Delta\Delta C_T}$ . (If the efficiency is less than one, then the new amplification efficiency value is used instead of 2.) For example, a ratio of 0.1, calculated in this manner, would indicate that the target sequence level in the test sample is one-tenth the level found in the calibrator sample. A direct comparison ( $\Delta C_T$ ) approach should allow the discrimination of 1-



fold differences in the quantities of target sequences in different samples with 95% confidence.

#### Specific Examples

##### Example 1:

Quantitative Measurement of *Stachybotrys chartarum* conidia using real time detection of PCR products with the TaqMan™ Fluorogenic probe system

Conidial stocks of the target fungus, e.g. *Stachybotrys chartarum*, and the reference target, e.g. *Geotrichum candidum*, were prepared to act as calibrator and internal standard, respectively.

Genomic DNAs were extracted from 20µl conidial suspensions using a glass bead milling and glass milk adsorption method. Briefly, this method involved mixing test and reference conidia suspensions (10µl ea.) with 0.3g of acid-washed glass beads (G-1277; Sigma, St. Louis, MO, USA) and 10µl, 100µl and 300µl, respectively, of glass milk suspensions, lysis buffer and binding buffer from an Elu-Quik DNA purification kit (Schleicher and Schuell, Keene, NH) in sterile 2ml conical bottom, screw cap tubes (506-636; PGC Scientifics, Gaithersburg, MD). The tubes were shaken in a mini beadbeater (Biospec Products, Bartlesville, OK) for one minute at maximum rate and DNAs were recovered in final

The TaqMan probes and primers were obtained from the custom oligonucleotide synthesis facility at PE-Applied Biosystems (Foster City, CA). TaqMan probes contained a TAMRA group conjugated to their 3'-terminal nucleotide and a FAM group linked to their 5'-terminal nucleotides as the quencher and reporter fluorochromes, respectively. For *Geotrichum candidum*, the forward primer is NS92F: 5'-CACCGCCCGTCGCTAC (SEQ ID NO:86), the reverse primer is GcandR1: 5'-AGAAAAGTTGCCCTCTCCAGTT (SEQ ID NO:87), and the probe is GeoP2: 5'-TCAATCCGGAAGCCTCACTAAGCCATT (SEQ ID NO:88). For *Stackybotrys chartarum*, the forward primer is StacF4 5'-TCCCAAACCCTTATGTGAACC (SEQ ID NO:186), the reverse primer is StacR5 5'-GTTTGCCCACTCAGAGAATACTGAAA (SEQ ID NO:187), and the probe is StacP2 5'-CTGCGCCCGGATCCAGGC (SEQ ID NO:188).

PCR reactions were prepared in 0.5ml thin-walled, optical grade PCR tubes (PE Applied Biosystems, Foster City, CA) by addition of the following components: 12.5µl of TaqMan Universal Master Mix (a 2x-concentrated, proprietary mixture of AmpliTaq Gold™ DNA polymerase, AmpErase® UNG, dNTPs with UTP, passive reference dye and optimized buffer components, PE Applied Biosystems, Foster City, CA); 2.5µl of mixture of forward and reverse primers (10nM each); 2.5µl of 400nM TaqMan probe; 2.5µl of 2mg/ml bovine serum albumin, fraction V (Sigma Chemical, St. Louis, MO) and 5µl of DNA template. Standard procedures for the operation of the model 7700, as described in the instrument's manual, were followed. This included the

use of all default program settings with the exception of reaction volume which was changed from 50 to 25 $\mu$ l. Thermal cycling conditions consisting of two min at 50°C, 10min at 95°C, followed by 40 cycles of 15s at 95°C and 1min at 60°C. Cycle threshold ( $C_T$ ) determinations, i.e. non-integer calculations of the number of cycles required for reporter dye fluorescence resulting from the synthesis of PCR products to become significantly higher than background fluorescence levels, were automatically performed by the instrument for each reaction using default parameters. Assays for *S. chartarum* (target) sequences and *G. candidum* (reference) sequences in the same DNA samples were performed in separate reaction tubes.

Quantification of *S. chartarum* conidia using the comparative  $C_T$  method was performed by first subtracting mean reference sequence  $C_T$  values from mean target sequence  $C_T$  values for both test samples and a pre-specified calibrator sample to obtain  $\Delta C_T$  values. Calibrator sample  $\Delta C_T$  values are then subtracted from  $\Delta C_T$  values of the test samples to obtain  $\Delta\Delta C_T$  values.

Calibrator samples were DNA extracts from mixtures of approximately  $2 \times 10^6$  *S. chartarum* (strain UMAH 6417) and  $2 \times 10^6$  *G. candidum* conidia. Test samples were mixed with the

known amount of *G. candidum* conidia and amplified by the known

quantities of *S. chartarum* conidia in the calibrator samples to obtain estimates of the absolute quantities of these conidia in the test samples.

Each series of DNA extracts was also analyzed using only *S. chartarum* target sequence assay results. In these calculations, calibrator sample  $C_T$  values were subtracted directly from corresponding test sample  $C_T$  values to obtain  $\Delta C_{T,STAE}$  values. These values were used in place of  $\Delta \Delta C_T$  values to determine the ratio of target sequences in the test and calibrator samples and to quantify *S. chartarum* conidia in the test samples as described above.

Air sampling was performed in rooms that had previously been occupied by infants diagnosed with PH from three in the Cleveland, Ohio area. Airborne particles were recovered in sterile BioSampler® vials (SKC Inc., Eighty Four, PA) connected to an AirCon-2 High Flow Sampler pump (Gilian Instrument Co., Clearwater, FL). Air samples were taken over an eight hour time period under passive conditions (i.e. with no activity occurring in the rooms) at a flow rate of 10 liters per min, for a total collection volume of 4.8m<sup>3</sup>. Two additional air samples were taken in the same manner over a twelve hour period from the basement of a home in the Cincinnati, Ohio area that was also determined to contain extensive *S. chartarum* growth. One of these samples was collected under passive conditions, as described above, while the other was collected under aggressive sampling conditions

(i.e. during and after a cleaning effort in the contaminated area).

Each of the BioSampler vessels was rinsed three times with 5ml of sterile distilled water. The pooled rinses from each vial were transferred to sterile 50ml capped test tubes (25330-50); Corning Inc., Corning, NY) and centrifuged for 15min at 1000 x g in a Sorval RC2 centrifuge using an SS-34 rotor (DuPont Instruments, Newton, CT). After carefully drawing off the top 13-14ml of the supernatants, the pelleted materials in each tube were resuspended in the remaining liquid and transferred to 2ml microfuge tubes (16-8100-27; PGC Scientific, Frederick, MD). These suspensions were centrifuged at 14,000rpm for 3min in an Eppendorf microcentrifuge (5415C; Brinkman Instruments, Westbury, NY) and the majority of the supernatants were again removed by pipetting. The pellets and small amounts of liquid remaining in each tube were adjusted to either 100 or 200µl with sterile distilled water.

Direct counts of putative *S. chartarum* conidia in 10µl aliquots of the recovered samples were made in a haemocytometer chamber. Separate counts of up to six aliquots of each samples were taken over the entire grid portion of the chamber and the mean counts were converted to cell

*chartarum* conidia were based on recognition of the characteristic size, shape and pigmentation of these conidia. Three additional 10 $\mu$ l aliquots of each recovered sample were mixed with *G. candidum* reference conidia and subjected to total genomic DNA extraction for subsequent analysis in the model 7700 as specified above.

Yields of target sequences extracted from these conidia samples and from calibrator samples were determined from their respective  $C_T$  results in the model 7700 and compared using both the  $\Delta\Delta C_T$  (including *Geotrichum* reference sequence data) and  $\Delta C_{T,STAC}$  (not including reference sequence data) versions of the comparative  $C_T$  method. Quantities of conidia estimated from these analyses were then compared with those determined from direct microscopic counts of the samples taken in a haemocytometer. As illustrated in Fig. 3, results obtained by the  $\Delta C_{T,STAC}$  analysis method and from direct counting showed good agreement for most the samples. In 13 of these 14 instances, the estimate of the  $\Delta C_{T,STAC}$  method was within a one-fold range of the direct counting result. The results further indicated that this level of presumed accuracy and precision (i.e. within a 50-200% range of direct counts) may be expected to occur in 95% of all analyses performed by the  $\Delta C_{T,STAC}$  method. Based on comparisons of results obtained by the  $\Delta\Delta C_T$  analysis method for the same samples (data not shown), it was estimated that this method would provide the same level of accuracy in only about 70% of all analyses. Conidia from each of the

different strains examined appeared to be quantified with similar degrees of precision and accuracy using the  $\Delta C_{T,STAC}$  analysis method.

The sensitivity of the TaqMan assay and the functional dynamic range of the  $\Delta C_{T,STAC}$  quantification method were further examined using ten-fold serial dilutions of *S. chartarum* strain UMAH 6417 conidia stock suspensions as test samples. These samples contained expected quantities of cells that ranged from 2 to 2,000 based on direct counting analyses of the starting stock suspensions. As shown in Fig. 4, the results of these analyses were again in good agreement with the expected results. Five of the eight measurements gave estimates that coincided with the expected quantities of conidia in the samples within the relative errors of the analyses. The mean results of these analyzers were within a one-fold range of the expected values in all instances. In one of these two experiments, a low level of signal (equivalent to an estimated mean quantity of 0.27 conidia) was observed in the negative control samples. Parallel samples taken from one of the two dilution series of conidia (cf. Experiment 2 in Fig. 4) were also subjected to DNA extractions in the absence of *Geotrichum* cells. Although these extract yielded slightly lower quantitative results than those

results were not statistically significant ( $P = 0.41$  and  $0.17$ ).

data not shown).

A final evaluation of the TagMan assay and  $\Delta C_{T,STAC}$  method was made by analyzing particulate samples collected from the inside air of four homes with known colonization of *S. chartarum*. TagMan-based results were again compared with those obtained by direct microscopic observations of the samples in a haemocytometer. As shown in Table 3, the two methods again gave similar mean determinations of the quantities of *S. chartarum* conidia in these samples with four of the five results agreeing within the relative errors of the TagMan analyses. No *S. chartarum* conidia were found in the fifth samples by direct microscopic observation, however, this sample also appeared to approach the detection limits of the TagMan assay with only two of the three replicate DNA extracts producing signals above background.

Example 1, Table 1. Quantification of *Stachybotrys chartarum* conidia recovered from indoor air samples by direct microscopic counting and the  $\Delta C_{T,STAC}$  method as determined from TagMan analysis.



Sample source	Sampling conditions <sup>a</sup>	Direct count estimate	4Ct, 57A <sup>c</sup> TagMan estimate	Relative error
		Conidia, m <sup>3</sup> air	Conidia, m <sup>3</sup> air	
Home 1 <sup>b</sup>	Passive	46 <sup>c</sup>	23 <sup>b</sup>	7.5-69
Home 2 <sup>b</sup>	Passive	15 <sup>c</sup>	14 <sup>c</sup>	5.2-37
Home 3 <sup>b</sup>	Passive	31 <sup>c</sup>	26 <sup>c</sup>	9.4-68
Home 4 <sup>b</sup>	Passive	0 <sup>c</sup>	2.2 <sup>c</sup>	0.3-19
Home 4 <sup>b</sup>	Aggressive	5600 <sup>c</sup>	4300 <sup>c</sup>	2660-7300

<sup>a</sup> Defined in Materials and Methods.

<sup>b</sup> Located in Cleveland, Ohio.

<sup>c</sup> Value based on a total air sample volume of 4.8m<sup>3</sup>.

<sup>d</sup> Located in Cincinnati, Ohio.

<sup>e</sup> Value based on a total air sample volume of 7.2m<sup>3</sup>.

### Example 2:

Quantification of fungus from dust using real time, fluorescent probe-based detection of PCR products

Dust samples from the home of an infant with pulmonary hemosiderosis in Cleveland, OH (Home 1) were collected using 37-mm filter cassettes, pore size 0.8  $\mu$ m, as the collection device. Samples were obtained from two rooms in the basement, the living room, and the dining room. Additional dust samples were obtained in a similar manner from the basement of a home in Cincinnati, OH (Home 2) containing a significant, but localized, growth of *S. chartarum* as determined by surface sample analysis. One sample was taken from the floor directly beneath the area of growth, a second from another location in the same room and a third from an

Total DNAs were extracted from dust samples using glass bead milling and glass milk adsorption method. Weighed dust samples were added directly to sterile 2 ml conical bottom, screw cap tubes (506-636; PGC Scientifics, Gaithersburg, MD), containing 0.3 g of glass beads (G-1277; Sigma, St. Louis, MO) and 100 and 300  $\mu$ l of lysis and binding buffer, respectively from an Elu-Quik DNA Purification Kit (Schleicher and Schuell, Keene, NH). Ten  $\mu$ l aliquots of a  $2 \times 10^7$  conidia/ml suspension of *G. candidum* in 0.5% Tween 20 were also routinely added to the tubes as a potential source of reference DNA sequences. Ten  $\mu$ l aliquots of *S. chartarum* conidia suspensions in water were also added as needed. The tubes were shaken in a mini beadbeater, (Biospec Products, Bartlesville, OH) for one minute at a maximum speed. To bind the DNA, 25  $\mu$ l of Elu-Quik glass milk suspension (Schleicher and Schuell, Keene, NH) was added to the samples and the tubes were placed on a minirotating mixer (Glas-Col, Terre Haute, IN) for 20 minutes. The samples were transferred to SPIN<sup>TM</sup> filter and catch tube assemblies (BIO 101, Vista CA) and centrifugation at 7500 X g for 1.5 min to remove binding and lysis buffers. The retained particulates, including glass milk with adsorbed nucleic acids, were washed twice in the filter cartridges with 0.5 ml Elu-Quik wash buffer and once with 0.5 ml Elu-Quik salt reduction buffer and centrifuged as above after each wash. Nucleic acids were desorbed from the glass milk particles by two successive washes with 100  $\mu$ l distilled

water and collected by centrifuging the washes into clean catch tubes. Calibrator samples, used in the analytical method as standards for the quantification of *S. chartarum* conidia in the test samples, contained  $2 \times 10^4$  *S. chartarum* and  $2 \times 10^5$  *G. candidum* conidia with no dust and DNA extractions from these samples was performed in the same manner.

PCR reactions were prepared in 0.5 ml thin-walled, optical grade PCR tubes (PE Biosystems, Foster City CA). Each reaction contained 12.5  $\mu$ l of "Universal Master Mix"- a 2X concentrated, proprietary mixture of AmpliTaq Gold™ DNA polymerase, AmpErase® UNG, dNTPs, passive reference dye and optimized buffer components (PE Biosystems, Foster City CA), 0.5  $\mu$ l of a mixture of forward and reverse primers at 50 mM each, 2.5  $\mu$ l of 400 nM TaqMan probe (PE Biosystems, Foster City, CA), 2.5  $\mu$ l of 2 mg/ml fraction V bovine serum albumin (Sigma Chemical, St. Louis, MO) and 2  $\mu$ l of autoclaved water. Five  $\mu$ l of purified DNA extract was added to complete the 25  $\mu$ l reaction mix.

The TaqMan probes and primers were obtained from the custom oligonucleotide synthesis facility at PE-Applied Biosystems (Foster City, CA). TaqMan probes contained a TAMRA group conjugated to their 3'-terminal nucleotide and a FAM group linked to their 5'-terminal nucleotides as the quencher

AGAAAAGTTGCCCTCTCCAGTT (SEQ ID NO:87), and the probe is GeoP2: 5'-TCAATCCGGAAGCCTCACTAAGGCATT (SEQ ID NO:88). For *Stachybotrys chartarum*, the forward primer is StacF4 5'-TCCCAAACCCCTTATGTGAACC (SEQ ID NO:186), the reverse primer is StacR5 5'-GTTTGCCTACTCAGAGATACTGAAA (SEQ ID NO:187), and the probe is StacP2 5'-CTGCGCCCCGATCCAGGC (SEQ ID NO:188).

Standard procedures for the operation of the model 7700, as described in the instrument's manual, were followed using all of the default program settings with the exception of reaction volume which was changed from 50 to 25  $\mu$ l. Thermal cycling conditions consisted of 2 minutes at 50°C, 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. Cycle threshold ( $C_T$ ) determinations were automatically performed by the instrument for each assay using default parameters. Assays for *S. chartarum* sequences and *G. candidum* sequences in the same DNA samples were performed in separate reaction tubes.

To quantify conidia the mean *S. chartarum* calibrator  $C_T$  value was subtracted from the mean *S. chartarum* sequence  $C_T$  values in the sample extracts to obtain  $\Delta C_{T,STAC}$  values. Ratios of target sequences in the test and calibrator samples were multiplied by the known quantities of *S. chartarum* conidia in the calibrator samples to obtain measurements of the quantities of these conidia in the test samples. Similar calculations were performed in parallel using *G. candidum* sequence  $C_T$  values from the same calibrator and test samples to

determine  $\Delta C_{T, \text{GEO}}$  values and quantities of these conidia in the test samples.

Then *G. candidum* sequence  $C_T$  values were subtracted from mean *S. chazartarum* sequence  $C_T$  values for both test and calibrator sample extracts to obtain  $\Delta C_T$  values. Calibrator sample  $\Delta C_T$  values were then subtracted from the test sample  $\Delta C_T$  values to obtain  $\Delta \Delta C_T$  values. These values were used in place of  $\Delta C_{T, \text{STAC}}$  values to determine the ratios of *S. chazartarum* target sequences in the test and calibrator samples and to quantify *S. chazartarum* conidia in the test samples as indicated above.

Variances of  $\Delta C_T$  were estimated from the results of the replicate extracts of each sample by:

$$S_{\Delta C_T}^2 = S_{\text{Target}}^2 + S_{\text{Ref}}^2 - 2rS_{\text{Target}}S_{\text{Ref}} \quad [1]$$

where  $S_{\text{Target}}$  and  $S_{\text{Ref}}$  are the standard deviations (SD) of the *S. chazartarum* and *G. candidum* assay results, respectively, and  $r$  is the correlation coefficient between these results.

Variances of  $\Delta \Delta C_T$  were estimated by  $S_{\Delta \Delta C_T}^2 = S_{\Delta C_T(\text{C})}^2 + S_{\Delta C_T(\text{S})}^2 \quad [2]$

where  $S_{\Delta C_T(\text{C})}$  is given by Equation [1] applied to the calibrator results, and  $S_{\Delta C_T(\text{S})}$  by Equation [1] applied to the test sample results. Since calibrator and test sample  $C_T$  values were

of the  $C_T$  for the calibrator. Variances of  $\Delta C_{T, \text{GEO}}$  values were calculated in the same manner. Standard errors of difference were determined from the appropriate standard deviation divided by the square root of the number of replicate observations (extractions), and confidence intervals for the differences were constructed using these standard errors.

With  $N_0$  representing the number of cells in the calibrator sample, the corresponding cell numbers in test samples were estimated by  $N_0 2^{-\Delta Y}$  [4], where  $\Delta Y$  was the estimator  $\Delta \Delta C_T$ ,  $\Delta C_{T, \text{STAC}}$ , or  $\Delta C_{T, \text{GEO}}$ . In this paper the term "relative error" refers the range implied by one standard deviation about  $\Delta Y$ , i.e.  $N_0 2^{-\Delta Y \pm S_{\Delta Y}}$ , in which  $S_{\Delta Y}$  is given by equation [2] or [3]. Confidence intervals were constructed around the estimated cell numbers by  $N_0 2^{-\Delta Y \pm t S_{\Delta Y} / \sqrt{3}}$ , where  $t$  is the appropriate Student  $t$ -value and three replicate extractions were used.

In method evaluation experiments, conidia quantities determined by the  $\Delta \Delta C_T$ ,  $\Delta C_{T, \text{STAC}}$ , or  $\Delta C_{T, \text{GEO}}$  methods (NT) were compared to "known" quantities of conidia added to the dust samples. The "known" quantities were determined from hemocytometer cell counts of three replicate aliquots (at least 400 total counts) of the conidia stock suspensions used for dilution and sample amendment. The "known" value for  $\Delta C_T$  ( $N_E$ ) was calculated from equation [4] based on the hemocytometer counts and dilution factors, and the differences:  $d = \Delta C_T - \text{known value}$  were evaluated via analysis

of variance to test the null hypothesis:  $d = 0$ . The 95% confidence level range for individual observations of  $d$  was constructed, assuming  $d$  to be normally distributed, and used to characterize the precision of a single estimate utilizing TaqMan quantification. Note that when antilogs are taken, the confidence interval describes lower and upper limits to the ratio  $N_T/N_H$ .

The direct enumeration of *Stachybotrys* conidia in dust samples was performed by weighing dust samples, suspending them in 0.5% Tween 20 to a concentration of 1 mg/ml and, with constant mixing of the suspensions, aliquots were applied to a hemocytometer chamber. Nine replicate aliquots, or fewer if this was sufficient to enumerate at least 400 conidia, were counted in this manner for each suspension. The volumes of the examined grids were used to calculate conidia numbers per ml of suspension and these values converted to numbers per mg of dust. For comparability with relative error of the TaqMan estimates, one standard deviation ranges for direct count estimates were calculated. Conidia were assumed to be randomly distributed within each grid. Under this assumption the corresponding relative error is a range such that the observed count represents an observation one standard deviation above or one standard deviation below a Poisson

enumeration estimates for *Stachybotrys* conidia in

dust samples taken from two contaminated homes were obtained by  $\Delta\Delta\text{CT}$  analyses of TagMan assay results and compared with the results of presumptive direct microscopic enumeration of these conidia. Mean estimates obtained from the TagMan assays fell within, or very close to the 0.24 to 1.04 range of direct counts that was predicted by the method evaluation experiments (Example 2, Table 1).

Example 2, Table 1. Quantities of *S. chartarum* conidia in home dust samples determined by LACT TagMan analysis and direct microscopic enumeration.

Location in Home	Proximity to Fungal Growth	<u><math>\Delta\Delta\text{CT}</math> TagMan Estimate</u>		<u>Direct count estimate</u>	
		Conidia/5mg dust	Relative Error <sup>a</sup>	Conidia/5mg dust	Relative Error <sup>a</sup>
Living Rm	Remote Room <sup>d</sup>	6	4 - 10	667	444 - 1000
Basement	Same Room	1100 <sup>f</sup>	-----	2333	1877 - 2901
Basement	Same Room	9200	7100 - 12000	26889	25215 - 28674
Dining Rm	Remote Room	560	420 - 740	1444	1096 - 1904
Basement	Same Room	23600	18300 - 31000	30444	29660 - 32340
Basement	Adjacent Room	300	260 - 340	776	534 - 1133
Basement	Same Room <sup>e</sup>	77200	56700 - 105000	68266	50737 - 55597
HVAC		1.7	0.3 - 11.2	556	357 - 866

a Home 1 located in Cleveland, OH

b Home 2 located in Cincinnati, OH

c Composite HVAC system dust as described in Methods section of text

d Small amount of fungal growth, no confirmed *Stachybotrys*

e Sample collected directly beneath area of fungal growth

Analyses of known numbers of *Stachybotrys* conidia over a range from  $2 \times 10^2$  to  $2 \times 10^4$  in the presence of 10 mg of composite HVAC system dust were found to provide 95% occurrence results within a range from 25% to 104% of expected values using this approach.

A second type of matrix effect that can affect PCR-based analyses of dust samples is the influence of PCR



inhibitory compounds. Retention of such compounds through the DNA extraction and purification procedures occurred in only one sample in this study. A simple procedure, involving the dilution and re-analysis of a DNA extract from this sample was used to identify this matrix effect and to obtain a corrected estimate of conidia quantities. This procedure should be generally applicable so long as the concentrations of target sequences in the samples are sufficiently high to still be detectable after the inhibitor's effects are negated by dilution. In practice, however, such follow-up analyses are only likely to be necessary when significant differences are observed in the reference sequence assay results of test and calibrator samples in the initial analyses of the samples.

Example 3: Evaluation of *Stachybotrys chartarum* in the House of an Infant with Pulmonary Hemorrhage: Quantitative Assessment Before, During and After Remediation

Air samples (Example 3, Table 1) were taken in a home under remediation for mold damage in two ways; either using a cassette filter (37mm with 0.8 mm filter) or with a BioSampler (SKC, Eighty Four, PA) connecting to an Aircon-2 High Flow Sampler pump (Gilian Instrument Co., Clearwater, FL) calibrated at a flow rate of 10 liter per min. These samples were taken for a period between 0 and 90 hrs at 10 liter per

filter).

Conidial stocks of the target fungus, i.e. *Stachybotrys chartarum*, and the reference target, i. e. *Geotrichum candidum*, were prepared to act as calibrator and internal standard, respectively.

Genomic DNAs were extracted from 20 µl conidial suspensions using a glass bead milling and glass milk adsorption method. Briefly, this method involved mixing test and reference conidia suspensions (10 µl ea.) with 0.3 g of acid-washed glass beads (G-1277; Sigma, St. Louis, MO) and 10 µl, 100 µl and 300 µl, respectively, of glass milk suspension, lysis buffer and binding buffer from an Elu-Quik DNA purification kit (Schleicher and Schuell, Keene, NH) in sterile 2 ml conical bottom, screw cap tubes (506-636; PGC Scientifics, Gaithersburg, MD). The tubes were shaken in a mini beadbeater (Biospec Products, Bartlesville, OK) for one minute at maximum rate and DNAs were recovered in final volumes of 200 µl distilled water after performing a slight modification of the small-scale protocol provided with the Elu-Quik purification kit.

The TaqMan probes and primers were obtained from the custom oligonucleotide synthesis facility at PE-Applied Biosystems (Foster City, CA). TaqMan probes contained a TAMRA group conjugated to their 3'-terminal nucleotide and a FAM group linked to their 5'-terminal nucleotides as the quencher and reporter fluorochromes, respectively. For *Geotrichum*

*Candidum*, the forward primer is NS92F: 5'-CACCGCCCGTCGCTAC (SEQ ID NO:86), the reverse primer is GcandR1: 5'-AGAAAAGTTGCCCTCTCCAGTT (SEQ ID NO:87), and the probe is GeoP2: 5'-TCAATCCGGAAGCCTCACTAAGCCATT (SEQ ID NO:88). For *Stachybotrys chartarum*, the forward primer is StacF4 5'-TCCCAAACCCCTTATGTGAACC (SEQ ID NO:186), the reverse primer is StacR5 5'-GTTTGCCACTCAGAGAATACTGAAA (SEQ ID NO:187), and the probe is StacP2 5'-CTGCGCCCGGATCCAGGC (SEQ ID NO:188).

PCR reactions were prepared in 0.5 ml thin-walled, optical grade PCR tubes (PE Applied Biosystems, Foster City CA) by addition of the following components: 12.5 µl of TaqMan Universal Master Mix, a 2 x concentrated, proprietary mixture of AmpliTaq Gold™ DNA polymerase, AmpErase® UNG, dNTPs with UTP, passive reference dye and optimized buffer components (PE Applied Biosystems, Foster City CA); 2.5 µl of a mixture of forward and reverse primers (10 nM each); 2.5 µl of 400 nM TaqMan probe; 2.5 µl of 2 mg/ml bovine serum albumin (fraction V, Sigma Chemical, St. Louis, MO) and 5 µl of DNA template. Standard procedures for the operation of the model 5700, as described in the instrument's manual, were followed. This included the use of all default program settings with the exception of reaction volume which was changed from 50 to 25 µl. Thermal cycling conditions consisting of two min at 50°C,

reporter dye fluorescence resulting from the synthesis of PCR products to become significantly higher than background fluorescence levels were automatically performed by the instrument for each reaction using default parameters. Assays for *S. chartarum* (target) sequences and *G. candidum* (reference) sequences in the same DNA samples were performed in separate reaction tubes.

Results of air sampling with either filters or BioSamplers indicated that the number of airborne *S. chartarum* spores in this PH house was low before the remediation began (Example 3, Table 1). The number of *S. chartarum* spores in the air, when the furnace blower was activated (typical condition for the winter months), increased by a factor of 17-47 in the living room. During demolition, the number of *S. chartarum* spores in the air increased by four orders of magnitude in the basement, about three orders of magnitude in the dining room and about two orders of magnitude in the upstairs bedroom (Example 3, Table 1). Thus this technology, under actual conditions, can detect the target fungus over four orders of magnitude.

Table 1. Results of air sampling for *S. chartarum* (S.c.) spores in the mold contaminated home.

Date	Sample Method	Location (Room = RM)	Sample Time (H)	S.c. Spores (#/m <sup>2</sup> air)
<b>Pre-remediation</b>				
12/29-30	Filter (Passive) <sup>1</sup> BioSampler (passive)	Living Rm	25.5	0.2
		Living Rm	25.5	0.3
12/30-31	Filter (active) <sup>2</sup> BioSampler (active)	Living Rm	24	9.3
		Living Rm	24	5.0
12/31	Filter (active) BioSample (active)	Dining Rm	90	0.1
		Dining Rm	90	1.7
12/31 - 1/4	Filter (active)	Easement	90	0.6
<b>During Remediation<sup>3</sup></b>				
1-19	Filter	Easement	6.6	$1.1 \times 10^3$
	BioSampler	Easement	6.6	$1.6 \times 10^3$
	Filter PMP <sup>4</sup>	Basement	6.5	$2.0 \times 10^3$
1-20	Filter	Dining Rm	6.25	$1.8 \times 10^3$
	BioSampler	Dining Rm	6.25	$2.7 \times 10^3$
	Filter PMP	Dining Rm	5.75	$4.0 \times 10^3$
1-21	Filter BioSampler	N. Bedrm	7.75	$0.1 \times 10^3$
		N. Bedrm	7.75	$0.1 \times 10^3$

1 "passive" means furnace blower off, furnace sealed and inoperable

2 "active" means furnace blower on, furnace operable.

#### EXAMPLE 4:

#### Identification and Quantification of *Helicobacter pylori*

Culturing of *Helicobacter pylori* from environmental sources continues to be an obstacle in detecting and enumerating this organism. Selection of primer and probe sequences for the *ureA* gene was performed based on comparative sequence analyses of 16 strains of *H. pylori* and other *Helicobacter* species. For *Helicobacter pylori*, the forward primer is HpylF1: 5'-GGGTATTGAGCCGATGTTTCCT (SEQ ID NO:223),

DNA was extracted from aliquots of ten-fold serial dilutions of *H. pylori* by EluQuick kits from Schleicher and Schuell, Inc. The cells were lysed, DNA bound to glass beads and washed with alcohol and salt reduction solutions followed by elution from filters with water. One set of extraction tubes, contained only *H. pylori*. A second set also received  $10^3$ /Ml *E. Coli*. Portions of some DNA extracts were subjected to agarose gel electrophoresis and GelStar staining. Yields of high molecular weight total DNA (appearing as bands on the 1.5% gels) were estimated by comparisons of their fluorescence signals with those of a series of known mass standards (Gibco/BRL) using a model S1 fluorimager (Molecular Dynamics).

The more commonly identified non-*pylori Helicobacter* species were tested with *H. pylori* primers and probe (Example 4, Table 1). Results show that when compared to the negative extraction control all of these species were also negative. All obtained  $C_T$  values in the, range of 37 to 39. A 40  $C_T$  is the lowest negative value obtainable. Counts of the bacteria were high. They ranged from  $10^7$  to  $10^8$  per assay. The *H. pylori* strain also was initially in this range with a  $C_T$  value was 15.

Example 4, Table 1

Bacteria	Dilution	Cells/TaqMan	C <sub>t</sub> Values
<i>Campylobacter jejuni</i>	10 <sup>0</sup>	8.75 x 10 <sup>6</sup>	36.55
	10 <sup>-1</sup>	8.75 x 10 <sup>5</sup>	36.86
	10 <sup>-2</sup>	8.75 x 10 <sup>4</sup>	38.78
<i>Helicobacter felis</i>	10 <sup>0</sup>	6.8 x 10 <sup>1</sup>	37.55
	10 <sup>-1</sup>	6.8 x 10 <sup>4</sup>	36.17
	10 <sup>-2</sup>	6.8 x 10 <sup>3</sup>	38.21
<i>Helicobacter hepoticus</i>	10 <sup>0</sup>	2.3 x 10 <sup>7</sup>	36.68
	10 <sup>-1</sup>	2.3 x 10 <sup>6</sup>	37.14
	10 <sup>-2</sup>	2.3 x 10 <sup>5</sup>	39.74
<i>Helicobacter mustelae</i>	10 <sup>0</sup>	1.9 x 10 <sup>6</sup>	34.66
	10 <sup>-1</sup>	1.9 x 10 <sup>7</sup>	36.37
	10 <sup>-2</sup>	1.9 x 10 <sup>6</sup>	37.65
<i>Helicobacter pylori</i>	100	2.1 x 10 <sup>7</sup>	14.93
	10 <sup>-1</sup>	2.1 x 10 <sup>6</sup>	18.23
	10 <sup>-2</sup>	2.1 x 10 <sup>5</sup>	21.45
	10 <sup>-3</sup>	2.1 x 10 <sup>4</sup>	25.24
	10 <sup>-4</sup>	2.1 x 10 <sup>3</sup>	32.73
	10 <sup>-5</sup>	2.1 x 10 <sup>2</sup>	34.63
	10 <sup>-6</sup>	2.1 x 10 <sup>1</sup>	35.24
	10 <sup>-7</sup>	2.1 x 10 <sup>0</sup>	39.58
Negative Extraction Control	-	-	37.65
Positive Calibrator Control	10 <sup>-1</sup>	9.8 x 10 <sup>5</sup>	17.3

Samples of serially diluted *H. pylori* cells spanning a 6 log concentration range were subjected to DNA extraction and TaqMan analysis.

Estimated cell quantities in the extracted samples ranged from 20 to 2 x 10<sup>6</sup> based on direct microscopic counts following staining with DAPI. Results from 5 replicate experiments showed a good correlation (r<sup>2</sup> = 0.99) between

were seen for two *Helicobacter pylori* strains. It was concluded that the TaqMan quantitative PCR method has the potential to provide accurate quantification of *H. pylori* cells in environmental samples.

Ten-fold dilutions of a single DNA extract are shown in Figure 4 along with the corresponding regression analysis. This curve is linear all the way to a negative  $C_T$  of 40. The  $R^2$  is 0.999. Counts that correspond to the initial dilution can be extrapolated for the other dilutions and can be included on the X-axis. Figure 4 shows a linear range from  $1.5 \times 10^5$  to 1.5 genome equivalents.

Example 4, Figure 4. Log (base 10) *H. pylori* counts per assay are plotted against the cycle threshold values.

#### CONCLUSIONS

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from



the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

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## WHAT IS CLAIMED IS:

1. A method of detecting and quantifying fungi and bacteria comprising obtaining a sequence of the fungus to be detected and quantified, extracting the DNA from a sample, subjecting said DNA to polymerase chain reaction and fluorescent probe analysis.

2. The method according to claim 1 wherein the fungi and bacteria are selected from the group consisting of *Absidia coerulea*, *Absidia glauca*, *Absidia corymbifera*, *Acremonium strictum*, *Alternaria alternata*, *Apophysomyces elegans*, *Saksena vasiformis*, *Aspergillus flavus*, *Aspergillus oryzae*, *Aspergillus fumigatus*, *Neosartoryta fischeri*, *Aspergillus niger*, *Aspergillus foetidus*, *Aspergillus phoenicus*, *Aspergillus nomius*, *Aspergillus ochraceus*, *Aspergillus ostianus*, *Aspergillus auricomus*, *Aspergillus parasiticus*, *Aspergillus sojae*, *Aspergillus restrictus*, *Aspergillus caesillus*, *Aspergillus conicus*, *Aspergillus sydowii*, *Aspergillus tamarii*, *Aspergillus terreus*, *Asperguters natus*, *Aspergillus versicolor*, *Aspergillus ustus*, *Aspergillus versicolor*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Cladosporium herbarum*, *Cladosporium sphaerospermum*, *Cladosporium*

amstelodami, *Eurotium chevalieri*, *Eurotium herbariorum*,  
*Eurotium rubrum*, *Eurotium repens*, *Geotrichum candidum* strain  
UAMH 7863, *Geotrichum candidum*, *Geotrichum klebahnii*,  
*Memnoniella echinata*, *Mortierella polycephala*, *Mortierella*  
*wolfii*, *Mucor mucedo*, *Mucor amphibiorum*, *Mucor circinelloides*,  
*Mucor heimalis*, *Mucor indicus*, *Mucor mucedo*, *Mucor racemosus*,  
*Mucor famosissimus*, *Rhizopus azygosporus*, *Rhizopus*  
*homothalicus*, *Rhizopus microsporus*, *Rhizopus oligosporus*,  
*Rhizopus oryzae*, *Myrothecium verrucaria*, *Myrothecium roridum*,  
*Paecilomyces lilacinus*, *Paecilomyces varioti*, *Penicillium*  
*freii*, *Penicillium verrucosum*, *Penicillium hirsutum*,  
*Penicillium alberechii*, *Penicillium aurantiogriseum*,  
*Penicillium polonicum*, *Penicillium viridicatum*, *Penicillium*  
*hirsutum*, *Penicillium breviscompactum*, *Penicillium chrysogenum*,  
*Penicillium griseofulvum*, *Penicillium glandicola*, *Penicillium*  
*coprophilum*, *Penicillium crustaceum*, *Penicillium egyptiacum*,  
*Penicillium crustosum*, *Penicillium citrinum*, *Penicillium*  
*sartoryi*, *Penicillium westlingi*, *Penicillium corylophilum*,  
*Penicillium decumbens*, *Penicillium echinulatum*, *Penicillium*  
*solitum*, *Penicillium schlerotigenum*, *Penicillium italicum*,  
*Eupenicillium expansum*, *Penicillium fellutanum*, *Penicillium*  
*charlesii*, *Penicillium janthinellum*, *Penicillium raperi*,  
*Penicillium madriti*, *Penicillium gladioli*, *Penicillium*  
*oxalicum*, *Penicillium roquefortii*, *Penicillium simplicissimum*,  
*Penicillium ochrochloron*, *Penicillium spinulosum*, *Penicillium*

*glabrum*, *Penicillium thomii*, *Penicillium pupurescens*,  
*Eupenicillium lapidosum*, *Rhizomucor miehei*, *Rhizomucor*  
*pusillus*, *Rhizomucor variabilis*, *Rhizopus stolonifer*,  
*Scopulariopsis asperula*, *Scopulariopsis brevicaulis*,  
*Scopulariopsis fusca*, *Scopulariopsis brumptii*, *Scopulariopsis*  
*chartarum*, *Scopulariopsis sphaerospora*, *Trichoderma*  
*aasperellum*, *Trichoderma hamatum*, *Trichoderma viride*,  
*Trichoderma harzianum*, *Trichoderma longibrachiatum*,  
*Trichoderma citroviride*, *Trichoderma atroviride*, *Trichoderma*  
*koningii*, *Ulocladium atrum*, *Ulocladium chartarum*, *Ulocladium*  
*botrytis*, *Wallemia sebi*, *Escherichia coli*, *Helicobacter*  
*pylorii*, *Penicillium verrucosum*, and *Stachybotrys chartarum*.

3. The method according to claim 2 wherein the fungi are selected from the group consisting of *Absidia coerulea/glauca*, the Forward Primer is NS92F: 5'-CACCGCCCGTCGCTAC (SEQ ID NO:1), the reverse primer is AcoerR1: 5'-TCTAGTTTGCCATAGTCTCTCCAG (SEQ ID NO:2), and the probe is NucF1: 5'-CCGATTGATGGTTATAGTGAACATATGGGATC (SEQ ID NO:3).

4. The method according to claim 2 wherein the fungi are selected from the group consisting of *Absidia conchyliformis*, the forward primer is NS92F: 5'-CAGCCGCGCCGTCGCTAC

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5 . The method according to claim 3 wherein the fungi are selected from the group consisting of *Acremonium strictum*, the forward primer is AstrcF1: 5'-CAACCCATTGTGAACTTACCAATC (SEQ ID NO:7), the reverse primer is AstrcR1: 5'-CGCCCCCTCAGAGAAATACGATT (SEQ ID NO:8), and the probe is AstrcP1: 5'-TCAGCGGCGCGGTGGCCTC (SEQ ID NO:9).

6 . The method according to claim 3 wherein the fungi are selected from the group consisting of *Alternaria alternata*, the forward primer is AaltrF1: 5'-EGCGGGCTGGAACCTC (SEQ ID NO:10), the reverse primer is AltrR1-1: 5'-GCAATTACAAAAGSTTTATGTTTGTGTA (SEQ ID NO:11), or the reverse primer is AaltrR1-2: 5'-TGCAATTACTAAAGSTTTATGTTTGTGTA (SEQ ID NO:12), and the probe is AaltrP1: 5'-TTACAGCCTTGCTGAATTATTCACCCCTGTCTTT (SEQ ID NO:13).

7 . The method according to claim 3 wherein the fungi are selected from the group consisting of *Apophysomyces elegans* and *Saksenea vasiformis*, the forward primer is NS92F: 5'-CACCGCCCGTCGCTAC (SEQ ID NO:14), the reverse primer is AelegR1: 5'-GACTCGAATGAGTTCTCGCTTC (SEQ ID NO:15), and the probe is AelegP1: 5'-TGGCCAAGACCAGAATATGGGATTTC (SEQ ID NO:16).

8 . The method according to claim 3 wherein the fungi are selected from the group consisting of *Aspergillus*

flavus/oryzae, the forward primer is AflavF1: 5'-CGAGTGTAGGGTTCTAGCGA (SEQ ID NO:17), the reverse primer is AflavR1: 5'-CCGGCGGCCATGAAT (SEQ ID NO:18), and the probe is AflavP1: 5'-TCCCACCCGTGTTTACTGTACCTTAGTTGCT (SEQ ID NO:19).

9 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Aspergillus fumigatus*, *Neosartorya fischeri*, the forward primer is AfumiF1: 5'-GCCCCCGCGTTTCGAC (SEQ ID NO:20), the reverse primer is AfumiR1: 5'-CCGTTGTTGAAAGTTTAACTGATTAC (SEQ ID NO:21), and the probe is AfumiP1: 5'-CCCGCGGAAGACCCCAACATG (SEQ ID NO:22).

10 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Aspergillus niger*/*foetidus*/*phoenicus*, the forward primer is AnigrF1: 5'-GCGGAGACCCCAACAC-3' (SEQ ID NO:23), the reverse primer is AnigrR1: 5'-TGTTGAAAGTTTAACTGATTGCATT-3' (SEQ ID NO:24), and the probe is AnigrP1: 5'-AATCAACTCAGACTGCACGCTTTCAGACAG (SEQ ID NO:25).

11 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Aspergillus nomius*, the forward primer is AflavF1: 5'-

12 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Aspergillus ochraceus*/*ostianus*/*auricomus*, the forward primer is *AcchrF1*: 5'-AACCTCCCACCCGTGTATACC-3' (SEQ ID NO:29), the reverse primer is *AcchrR1*: 5'-CCGCGAGCGCGCTGTG-3' (SEQ ID NO:30), and the probe is *AcchrP1*: 5'-ACCTTGTTGCTTCGGCGAGCCC (SEQ ID NO:31).

13 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Aspergillus parasiticus*/*sojae*, the forward primer is *AflavF1*: 5'-CGAGTGTAGGGTTCTAGCGA-3' (SEQ ID NO:32), the reverse primer is *AparaR3*: 5'-GCCCGGGGCTGACG-3' (SEQ ID NO:33), and the probe is *AflavP1*: 5'-TCCCACCCCGTGTCTTACTGTACCTTAGTTGCT (SEQ ID NO:34).

14 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Aspergillus restrictus*/*caesillus*/*conicus*, the forward primer is *ArestF2*: 5'-CGGGCCCGCCTTCAT-3' (SEQ ID NO:35), the reverse primer is *ArestR1*: 5'-GTTGTTGAAAGTTTAAAGATTTTCT (SEQ ID NO:36), and the probe is *ArestP1*: 5'-CCCGCCGGAGACTCCAACATTG (SEQ ID NO:37).

15 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Aspergillus* sp. 11, the forward primer is *Aspd11F*: 5'-CAACCTCCACCCGTCAG-3' (SEQ ID NO:38), the reverse primer is *versR1*: 5'-



CCATTGTTGAAAGTTTGTGACTGATTTTA (SEQ ID NO:39), and the probe is  
versP1: 5'-AGACTGCATCACTCTCAGGCATGAAGTTCAG (SEQ ID NO:40).

16 . The method according to claim 2 wherein the  
fungi are selected from the group consisting of *Aspergillus*  
*tamarii*, the forward primer is AflavF1: 5'-  
CGAGTGTAGGGTTCTAGCGA (SEQ ID NO:41), the reverse primer is  
AtamaF1: 5'-CCCGGGGGCCTTAA (SEQ ID NO:42), and the probe is  
AflavF1: 5'-TCCCACCCGTGTTTACTGTACCTTAGTTGCT (SEQ ID NO:43).

17 . The method according to claim 2 wherein the  
fungi are selected from the group consisting of *Aspergillus*  
*terreus*, the forward primer is AterrF1: 5'-  
TTACCGAGTGCGGGTCTTTA (SEQ ID NO:44), the reverse primer is  
AterrF1: 5'-CGGCGGCCAGCAAC (SEQ ID NO:45), and the probe is  
AterrF1: 5'-AACCTCCCACCCGTGACTATTGTACCTTG (SEQ ID NO:46).

18 . The method according to claim 2 wherein the  
fungi are selected from the group consisting of *Aspergillus*  
*ustus*, the forward primer is AustsF1: 5'-  
GATCATTACCGAGTCCAGGCT (SEQ ID NO:47), the reverse primer is  
AustsF1: 5'-GCCGAAGCAACGTTGGTC (SEQ ID NO:48), and the probe  
is AustsF1: 5'-CCCCGGGGCAGGCCTAACC (SEQ ID NO:49).

versicolor, the forward primer is AversF2: 5'-CGGCGGGGAGCCCT (SEQ ID NO:50), the reverse primer is versR1: 5'-CCATTGTTGAAAGTTTGGACTGATTTTA (SEQ ID NO:51), and the probe is versP1: 5'-AGACTGCAATCACTCTCAGGCATGAAGTTCAG (SEQ ID NO:52).

20 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Chaetomium globosum*, the forward primer is CglobF1: 5'-CCGCAGGCCCTGAAAAG (SEQ ID NO:53), the reverse primer is CglobR1: 5'-CGCGGCGCGACCA (SEQ ID NO:54), and the probe is CglobP1: 5'-AGATGTATGCTACTACGCTCGGTGCGACAG (SEQ ID NO:55).

21 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Cladosporium cladosporioides* the Type 1, the forward primer is Cclad1F1: 5'-CATTACAAGTGACCCCGGTCTAAC (SEQ ID NO:56), the reverse primer is CcladR1: 5'-CCCCGGAGGCAACAGAG (SEQ ID NO:57), and the probe is CcladP1: 5'-CCGGGATGTTTCATAACCCCTTTGTTGTCC (SEQ ID NO:58); and for Type 2 the forward primer is Cclad2F1: 5'-TACAAGTGACCCCGGCTACG (SEQ ID NO:59), the reverse primer is CcladR1: 5'-CCCCGGAGGCAACAGAG (SEQ ID NO:60), and the probe is CcladP1: 5'-CCGGGATGTTTCATAACCCCTTTGTTGTCC (SEQ ID NO:61).

22 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Cladosporium herbarum*, the forward primer is CherbF1: 5'-AAGAACGCCCGGGCTT

(SEQ ID NO:62), the reverse primer is CherbR1: 5'-CGCAAGAGTTTGAAGTGTCCAC (SEQ ID NO:63), and the probe is CherbP1: 5'-CTGGTTATTCATAACCCTTTGTTGTCCGACTCTG (SEQ ID NO:64).

23 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Cladosporium sphaerospermum*, the forward primer is CsphaF1: 5'-ACCGGCTGGGTCTTTG (SEQ ID NO:65), the reverse primer is CsphaR1: 5'-GGGTTGTTTACGGCGTG (SEQ ID NO:66), and the probe is CsphaP1: 5'-CCCGCGGCACCCTTTAGCGA (SEQ ID NO:67).

24 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Conidiobolus coronatus/incongruus*, the forward primer is NS92F: 5'-CACCGCCCGTCGCTAC (SEQ ID NO:68), the reverse primer is ConiR1: 5'-TGACCAAGTTTGACCAATTTCTCTA (SEQ ID NO:69), and the probe is ConiP1: 5'-ATGTTTAGTGAGGCCTCTGGATTGAAGCTT (SEQ ID NO:70).

25 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Cunninghamella elegans*, the forward primer is NS91F: 5'-CACCGCCCGTCGCTAC (SEQ ID NO:71), the reverse primer is CunR1: 5'-AATCTAGTTTGCCATAGTCTCCTCA (SEQ ID NO:72), and the probe is

26 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Emericella nidulans*/*rugulosa*/*quadrilineata*, the forward primer is AversF1: 5'-CAACCTCCCACCCGTGAC (SEQ ID NO:74), the reverse primer is AniduR1: 5'-CATTGTGAAAGTTTGAAGTATTGTTGT (SEQ ID NO:75), and the probe is versP1: 5'-AGACTGCATCACTCTCAGGCATGAAGTTCAG (SEQ ID NO:76).

27 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Eurotium mstelodami*/*chevalieri*/*herbariorum*/*rubrum*/*repens*, the forward primer is EamstF1: 5'-GTGGCGGCACCATGTCT (SEQ ID NO:77), the reverse primer is EamstR1: 5'-CTGGTTAAAAAGATTGGTTGCGA (SEQ ID NO:78), and the probe is EamstP1: 5'-CAGCTGGACCTACGGGAGCGGG (SEQ ID NO:79).

28 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Epicoecum nigrum*, the forward primer is EnigrF1: 5'-TTGTAGACTTCGGTCTGCTACCTCTT (SEQ ID NO:80), the reverse primer is EnigrR1: 5'-TGCAACTGCAAAGGGTTTGAAT (SEQ ID NO:81), and the probe is EnigrP1: 5'-CATGTCTTTTGAGTACCTTCGTTTCTCTCGGC (SEQ ID NO:82).

29 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Geotrichum*

candidum strain UAMH 7863, the forward primer is GeoF1: 5'-GATATTTCTTGTGAATGCGAGAAGTGA (SEQ ID NO:83), the reverse primer is GeoR1: 5'-TTGATTGCGAAATTTTAGAAGAGCAAA (SEQ ID NO:84), and the probe is GeoP1: 5'-CAATTCCAAGAGAGAAACAACGCTCAAACAAG (SEQ ID NO:85).

30 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Geotrichum candidum*, the forward primer is NS92F: 5'-CACC GCCCGTCGCTAC (SEQ ID NO:86), the reverse primer is GcandF1: 5'-AGAAAAGTTGCCCTCTCCAGTT (SEQ ID NO:87), and the probe is GeoP2: 5'-TCAATCCGGAAGCCTCACTAAGCCATT (SEQ ID NO:88).

31 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Geotrichum klebahnii*, the forward primer is NS92F: 5'-CACC GCCCGTCGCTAC (SEQ ID NO:89), the reverse primer is GklebF1: 5'-AAAAGTCGCCCTCTCTGTC (SEQ ID NO:90), and the probe is GeoP2: 5'-TCAATCCGGAAGCCTCACTAAGCCATT (SEQ ID NO:91).

32 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Memnoniella echinata*, the forward primer is StacF4 5'-TCTTAAAGCCTTCTCTCTGTC (SEQ ID NO:92), the reverse primer is

33 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Mortierella polycephala*/*wolfii*, the forward primer is NS92F: 5'-CACCGCCCGTCGCTAC (SEQ ID NO:95), the reverse primer is MortR1: 5'-TGACCAAGTTTGGATAACTTTTCAG (SEQ ID NO:96), and the probe is MortP1: 5'-CTTAGTGAGGCTTTCGATTGGATCTAGGCA (SEQ ID NO:97).

34 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Mucor mucedo*, the forward primer is NS92F: 5'-CACCGCCCGTCGCTAC (SEQ ID NO:98), the reverse primer is MmucR1: 5'-CTAAATAATCTAGTTTGCCATAGTTTTCG (SEQ ID NO:99), and the probe is MucP1: 5'-CCGATTGAATGGTTATAGTGAGCATATGGGATC (SEQ ID NO:100).

35 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Mucor amphibiorum*/*circinelloides*/*heimalis*/*indicus*/*mucedo*/*racemosus*/*ramosissimus* and *Rhizopus azygosporus*/*homothalicus*/*microsporus*/*oligosporus*/*oryzae*, the forward primer is NS92F: 5'-CACCGCCCGTCGCTAC (SEQ ID NO:101), the reverse primer is MucR1-1: 5'-CCTAGTTTGCCATAGTTTCAGCAG (SEQ ID NO:102), and the probe is MucP1: 5'-CCGATTGAATGGTTATAGTGAGCATATGGGATC (SEQ ID NO:103).

36 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Myrothecium*

*verrucaria/roridum*, the forward primer is MyroF1: 5'-AGTTTACAAACTCCCAAACCCTTT (SEQ ID NO:104), the reverse primer is MyroR1: 5'-GTGTCACCTCAGAGGAGAAAACCA (SEQ ID NO:105), and the probe is MyroP1: 5'-CGCCTGGTTCCGGGCCC (SEQ ID NO:106).

37 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Paecilomyces lilacinus*, the forward primer is lilaF1: 5'-CCCACTGTGAACCTTACCTCAG (SEQ ID NO:107), the reverse primer is PlilaR1: 5'-GCTTGTGCAACTCAGAGAAGAAAT (SEQ ID NO:108), and the probe is PlilaP1: 5'-CCGCCCGCTGGGCGTAATG (SEQ ID NO:109).

38 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Paecilomyces variotii*, the forward primer is PvariF1: 5'-CCCGCCGTGGTTCAC (SEQ ID NO:110) or the forward primer is PvariF2: 5'-CGAAGACCCCTGGAACG (SEQ ID NO:111), and the reverse primer is PvariR1: 5'-GTTGTTGAAAGTTTTAATTGATTGATTGT (SEQ ID NO:112), and the probe is PvariP1: 5'-CTCAGACGGCAACCTTCCAGGTA (SEQ ID NO:113).

39 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium*

GAAAGTTTTAAATAATTTATATTTTCACTCAGAGTT (SEQ ID NO:115), and the probe is PenP2: 5'-CGCGCCCGCCGAAGACA (SEQ ID NO:116).

40 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium aurantiogriseum/polonicum/viridicatum/freii*, the forward primer is PauraF2: 5'-ACCGAGTGAGGGCCCTT (SEQ ID NO:117), the reverse primer is PauraR6: 5'-CCCGGGGGCCAGTA (SEQ ID NO:118), and the probe is PenP3: 5'-TCCAACTCCCACCCGIGTTTATTT (SEQ ID NO:119).

41 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium brevicompactum\*/alberechii*, the forward primer is PbrevF1: 5'-CCTTGTTGCTTCGGCGA (SEQ ID NO:120), the reverse primer is PbrevR2: 5'-TCAGACTACAATCTTCAGACAGAGTTCTAA (SEQ ID NO:121), and the probe is PbrevP1: 5'-CCTGCCTTTTGGCTGCCGGG (SEQ ID NO:122).

42 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium chrysogenum/griseofulvum/glandicola/coprophilum/expansum* and *Eupenicillium crustaceum/egyptiacum*, the forward primer is PchryF1: 5'-CGGGCCCGCCTTAAC (SEQ ID NO:123), the reverse primer is PchryR1-1: 5'-GAAAGTTTTAAATAATTTATATTTTCACTCAGAGTA (SEQ ID NO:124) or the reverse primer is PchryR2-1: 5'-



GAAAGTTTTAAATAATTATATTTTCACTCAGACCA (SEQ ID NO:125), and the probe is PenP2: 5'-CGGCGCCGCGCGAAGACA (SEQ ID NO:126).

43 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium citrinum*/*sartoryi*/*westlingi*, the forward primer is PcitrF1: 5'-CCGTGTTGCCCGAACCTA (SEQ ID NO:127), the reverse primer is PcitrR1: 5'-TTGTTGAAAGTTTAACTAATTCGTTATAG (SEQ ID NO:128), and the probe is PcitrP2: 5'-CCCCTGAACGCTGTCTGAAGTTGCA (SEQ ID NO:129).

44 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium corylophilum*, the forward primer is PcoryF1: 5'-GTCCAACCTCCCACCCA (SEQ ID NO:130), the reverse primer is PcoryR3-1: 5'-GCTCAGACTGCAATCTTCAGACTGT (SEQ ID NO:131), and the probe is PcoryP1: 5'-CTGCCCTCTGGCCCGCG (SEQ ID NO:132).

45 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium decumbens*, the forward primer is PdecuF3: 5'-GGCCTCCGTCCCTCCTTG (SEQ ID NO:133), the reverse primer is PdecuR3: 5'-AAAAGATTGATGTGTTCCGCCAG (SEQ ID NO:134), and the

47 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium expansum*/*coprophilum*, the forward primer is PauraF2: 5'-ACCGAGTGAGGGCCCTT (SEQ ID NO:139), the reverse primer is PchryR6: 5'-CCCGGCGGGCAGTT (SEQ ID NO:140), and the probe is PenP3: 5'-TCCAACCTCCCCACCCGTGTTTATTT (SEQ ID NO:141).

48 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium fellutanum*/*charlesii*, the forward primer is PfellF1: 5'-AACCTCCCACCCGTGTATACTTA (SEQ ID NO:142), the reverse primer is PfellR1: 5'-CTTATCGCTCAGACTGCAGGTA (SEQ ID NO:143), and the probe is PfellP1: CGGTTGCCCCCCGGGG (SEQ ID NO:144).

49 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium janthinellum/raperi*, the forward primer is PjantF2: 5'-GTAATCTTGTATTCGTACCA (SEQ ID NO:145), the reverse primer is PjantR2: 5'-TTGAAAGCTTTAACTGATTTAGCTAATCG (SEQ ID NO:146), and

the probe is PjantP2: 5'-TGCAATCTTCAGACAGCGTTCAGGG (SEQ ID NO:147).

50 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium madriti*/*gladioli*, the forward primer is PauraF1: 5'-CGGGCCCGCCTTTAC (SEQ ID NO:148), the reverse primer is PchryR1-1: 5'-GAAAGTTTTAAATAATTTATATTTTCACTCAGAGTA (SEQ ID NO:149) or the reverse primer is PchryR2-1: 5'-GAAAGTTTTAAATAATTTATATTTTCACTCAGACCA (SEQ ID NO:150), and the probe is PenP2: 5'-CGCGCCCGCCGAAGACA (SEQ ID NO:151).

51 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium oxalicum*, the forward primer is PoxalF1: 5'-GGGCCCGCCTCAGG (SEQ ID NO:152), the reverse primer is PoxalR1: 5'-GTTGTTGAAAGTTTTAACTGATTTAGTCAAGTA (SEQ ID NO:153), and the probe is PoxalP1: 5'-ACAAGAGTTCGTTTGTGTGTCTTCGGCG (SEQ ID NO:154).

52 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium roquefortii*, the forward primer is PchryF1: 5'-CGGGCCCGCCTTAAC

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53 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium simplicissimum*/ochrochloron, the forward primer is PsimpF1-1: 5'-AACCTCCCACCCGTGTTGATT (SEQ ID NO:158), the reverse primer is PsimpR2-1: 5'-GAGATCCGTTGTTGAAAGTTTTATCTG (SEQ ID NO:159) or the reverse primer is PsimpR3-1: 5'-GAGATCCGTTGTTGAAAGTTTAAACAG (SEQ ID NO:160), and the probe is PsimpP1: 5'-CCGCCTCACGGCCGGC (SEQ ID NO:161).

54 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium spinulosum*/glabrum/thomii/pupurescens and *Eupenicillium lapidosum*, the forward primer is PspinF1: 5'-GTACCTTGTTGCTTCGGTGC (SEQ ID NO:162), the reverse primer is PspinR1: 5'-CGTTGTTGAAAGTTTAACTTATTTAGTTTAT (SEQ ID NO:163), and the probe is PspinP1: 5'-TCCGCGCGCACCGGAG (SEQ ID NO:164).

55 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Rhizomucor miehei*/pusillus/variabilis, the forward primer is NS92F: 5'-CACCSCCCGTCGCTAC (SEQ ID NO:165), the reverse primer is RmucR1: 5'-GTAGTTTGCCATAGTTCGGCTA (SEQ ID NO:166), and the probe is RmucP1: 5'-TTGAATGGCTATAGTGAGCATATGGGAGGCT (SEQ ID NO:167).

56 . The method according to claim 2 wherein the

fungi are selected from the group consisting of *Rhizopus stolonifer*, the forward primer is NS92F: 5'-CACCGCCCGTCGCTAC (SEQ ID NO:168), the reverse primer is RstolR1: 5'-GCTTAGTTTGGCATAGTTCTCTAACAA (SEQ ID NO:169), and the probe is MucP1: 5'-CCGATTGAATGGTTATAGTGAGCATATGGGATC (SEQ ID NO:170).

57 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Scopulariopsis asperula*, the forward primer is SCbrvF1: 5'-CCCCTGCGTAGTAGATCCTACAT (SEQ ID NO:171), the reverse primer is SCasprR1: 5'-TCCGAGGTCAAACCATGAGTAA (SEQ ID NO:172) and the probe is ScopP1: 5'-TCGCATCGGGTCCCGGCG (SEQ ID NO:173).

58 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Scopulariopsis brevicaulis/fusca*, the forward primer is SCbrvF1: 5'-CCCCTGCGTAGTAGATCCTACAT (SEQ ID NO:174), the reverse primer is SCbrvR1: 5'-TCCGAGGTCAAACCATGAAATA (SEQ ID NO:175), and the probe is ScopP1: 5'-TCGCATCGGGTCCCGGCG (SEQ ID NO:176).

59 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Scopulariopsis brumptii*, the forward primer is SCbrmF1: 5'-

60 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Scopulariopsis chartarum*, the forward primer is SCchrF1: 5'-CCCCCTGCGTAGTAGTAAAGC (SEQ ID NO:180), the reverse primer is SCchrR1: 5'-TCCGAGGTCAAACCATCAAG (SEQ ID NO:181), and the probe is ScopP1: 5'-TCGCATCGGGTCCCGGGC (SEQ ID NO:182).

61 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Scopulariopsis sphaerospora*, the forward primer is SCsphF1: 5'-CCCCCTGCGTAGTAGTTTACAA (SEQ ID NO:183), the reverse primer is SCsphR1: 5'-CCGAGGTCAAACCATCAAG (SEQ ID NO:184), and the probe is ScopP1: 5'-TCGCATCGGGTCCCGGGC (SEQ ID NO:185).

62 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Stachybotrys chartarum*, the forward primer is StacF4 5'-TCCCAAACCCCTTATGTGAACC (SEQ ID NO:186), the reverse primer is StacR5 5'-GTTTGCCACTCAGAGAATACTGAAA (SEQ ID NO:187), and the probe is StacP2 5'-CTGCGCCCGGATCCAGGC (SEQ ID NO:188).

63 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Trichoderma asperellum/hamatum*, the forward primer is TasprF1: 5'-CCGGAACCAATGTGATGCT (SEQ ID NO:189), the reverse primer is TasprR2-1: 5'-GGACTACAGAAAGAGTTTGGTTGCTT (SEQ ID NO:190), and

the probe is TridP1: 5'-CCAAACTGTTGCCTCGGCGGG (SEQ ID NO:191).

64 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Trichoderma asperellum*/hamatum/viride\*, the forward primer is TasprF1: 5'-CCCAAACCCAATGTGAACGT (SEQ ID NO:192), the reverse primer is TasprR1: 5'-TTTGCTCAGAGCTGTAAGAAATACG (SEQ ID NO:193), and the probe is TridP1: 5'-CCAAACTGTTGCCTCGGCGGG (SEQ ID NO:194).

65 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Trichoderma harzianum*, the forward primer is TharzF1: 5'-TTGCCTCGGCGGGAT (SEQ ID NO:195), the reverse primer is TharzR1: 5'-ATTTTCGAAACGCCTACGAGA (SEQ ID NO:196), and the probe TharzP1: 5'-CTGCCCCGGGTGCGTCG (SEQ ID NO:197).

66 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Trichoderma longibrachiatum*/citroviride, the forward primer is TlongF1: 5'-TGCCTCGGCGGGATTC (SEQ ID NO:198), the reverse primer is TlongR1: 5'-CCAGAAAGGCTCAGATCAAAAT (SEQ ID NO:199), and the probe is TlongP1: 5'-TCGCAGCCCCGGATCCCA (SEQ ID NO:200).

67 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Trichoderma reesei*, the forward primer is TreseF1: 5'-TGCCTCGGCGGGATTC (SEQ ID NO:201), the reverse primer is TreseR1: 5'-CCAGAAAGGCTCAGATCAAAAT (SEQ ID NO:202), and the probe is TreseP1: 5'-TCGCAGCCCCGGATCCCA (SEQ ID NO:203).

5'-CCCAAACCCCAATGTGAACCA (SEQ ID NO:201), the reverse primer is IviriR1: 5'-TCCGCGAGGGGACTACAG (SEQ ID NO:202), and the probe is TrldP1: 5'-CCAAACTGTGTGCTTCGGCGGG (SEQ ID NO:203).

68 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Ulocladium atrum/chartarum*, the forward primer is UatrmF1: 5'-GCGGGGTGGCATCCTT (SEQ ID NO:204), the reverse primer is UatrmR1: 5'-TTGTCTATGTGTGGGCGAA (SEQ ID NO:205), and the probe is UloP1: 5'-TGAATTATTCAACCGTGTCTTTTGGCTACTTCT (SEQ ID NO:206).

69 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Ulocladium botrytis*, the forward primer is UbotrF1: 5'-CCCCCAGCAGTGCCTT (SEQ ID NO:207), the reverse primer is UbotrR1: 5'-CTGATTGCAATTACAAAAGGTTTATG (SEQ ID NO:208), and the probe is UloP1: 5'-TGAATTATTCAACCGTGTCTTTTGGCTACTTCT (SEQ ID NO:209).

70 . The method according to claim 2 wherein the fungi are selected from the group consisting of *Wallemia sebi*, the forward primer is WsebiF1: 5'-GGCTTAGTGAATCCTTCGGAG (SEQ ID NO:210), the reverse primer is WsebiR1: 5'-GTTTACCCAACTTTCAGTCCA (SEQ ID NO:211), and the probe is WsebiP1: 5'-TGTCGGGTTCGGGCTCAATAG (SEQ ID NO:212).



72 . The method according to claim 2 wherein the bacteria are selected from the group consisting of Escherichia coli, the forward primer is uidAF1: 5'-GGGCAGGCCAGCGTATC (SEQ ID NO:219), the reverse primer is uidAR1: 5'-CCCAIACCTTTGCCGTAAATGA (SEQ ID NO:220) or the reverse primer is uidAR2: 5'-CGTACACTTTTCCCGGCAAT (SEQ ID NO:221) and the probe is uidAP1: 5'-TGCTGCGTTTTCGATGCGGTCA (SEQ ID NO:222).

73 . The method according to claim 2 wherein the  
nucleotides are selected from the group consisting of  
*Helicobacter pylori*, the forward primer is HpylF1: 5'-  
GGGTAATTGAGCGGATGTTTCCT (SEQ ID NO:223), the reverse primer is  
HpylR1: 5'-GGTCTTCTTGGCTGCTTCTTCTT (SEQ ID NO:224)

74. The method according to claim 1 wherein the label is a fluorescent label.

75. The method according to claim 1 wherein fungi are detected and quantitated using PCR, hybridization, or other molecular techniques.

76. The method according to claim 2 wherein the primer and probes are used of determining the cell quantities of fungi and bacteria.

77. The method according to claim 2 wherein the fungi are selected from the group consisting of *Aspergillus caespitosus* the forward primer is AcaesF1:  
5'-CTCCCACCCGTGAATACCTT the reverse primer is AcaesR1:  
5'-GGCTCAGACGCAACTCTCAAT and the probe is AcaesP1:  
5'-CACTGTTGCTTCGGCGAGGAGCC.

78. The method according to claim 2 wherein the fungi are selected from the group consisting of *Aspergillus candidus*, the forward primer is AcandF1: 5'-TTACCGAGTGAGGGTTTCTCTGA the reverse primer is Acand R1: 5'-ACAGTGTTCGTGTTGGGGTCTT and the probe is PsimpP1: 5'-CCGCCTCACGGCCGCC.

79. The method according to claim 2 wherein the fungi are selected from the group consisting of *Aspergillus cervinus*,

the forward primer is AcervF1: 5'-CCACCCGTGCTATTGTACCTTT  
the reverse primer is AcervR1-1: 5'-CAACTCAGACTGCAATTCAGAACTGT  
and the probe is AfumiP2: 5'-TTCTCGGCGGGCGCGG.

80. The method according to claim 2 wherein the fungi are selected from the group consisting of *Aspergillus clavatus*, the forward primer is AclavF1: 5'-CCCGCCGTCTTCGGA the reverse primer is AclavR1: 5'-CCGTTGTTGAAAGTTTAACTGATTATG, and the probe is AfumiP1: 5'-CCCGCCGAAGACCCCAACATG.

81. The method according to claim 2 wherein the fungi are selected from the group consisting of *Aspergillus flavipes*, the forward primer is AflvpF1: 5'-CCACCCGTGACTACTGTACCAC, the reverse primer is AflvpR1: 5'-CCGGCGGCCAGCTAG, the reverse primer is AflvpR2: 5'-AGGCTTTCAGAAACAGTGTTCG, and the probe is AspP1: 5'-TTGCTTCGGCGGGGCC.

82. The method according to claim 2 wherein the fungi are selected from the group consisting of *Aspergillus niveus*, the forward primer is, AniveF1: 5'-ACCCGTGCCTATTGTACCTT, the reverse primer is AniveR1: 5'-TGCAAAACAATCACAATCAGACAC, and the probe is AspP1: 5'-TTGCTTCGGCGGGGCC.

the forward primer is AniveF1: 5'-ACCCGTGCCTATTGTACCTT, the

reverse primer is AochrR2-1: 5'-CGGCGAGCGCTGTtCC, and the probe is AochrP1: 5'-ACCTTGTTGCTTCGGCGAGCCC.

84. The method according to claim 2 wherein the fungi are selected from the group consisting of *Aspergillus ostianus*, the forward primer is AochrF1: 5'-AACCTCCCACCCGTGTATACC, the reverse primer is AostiR1-1: 5'-CGGCGAGCGCTGTTCT, and the probe is AochrP1: 5'-ACCTTGTTGCTTCGGCGAGCCC.

85. The method according to claim 2 wherein the fungi are selected from the group consisting of *Aspergillus paradoxus*, the forward primer is AparF1: 5'-CGGGGGGCTTACGCT, the reverse primer is AparR1-1: 5'-GACTGCAACTTCATACAGAGTTGGT, and the probe is PenP2: 5'-CGCGCCCGCCGAAGACA.

86. The method according to claim 2 wherein the fungi are selected from the group consisting of *Aspergillus penicillioides*, the forward primer is ApeniF2: 5'-CGCCGGAGACCTCAACC, the reverse primer is ApeniR2: 5'-TCCGTTGTTGAAAGTTTAAAGCA: and the probe is ApeniP2: 5'-TGAACACTGTCTGAAGGTTGCAGTCTGAGTATG.

87. The method according to claim 2 wherein the fungi are selected from the group consisting of *Aspergillus sclerotiorum*, the forward primer is AcircF1: 5'-ATTACTGAGTGAGGGTCCCTCG, the reverse primer is AsclrR1:

5'-CCTAGGSAGGGGGGTTTGA, and the probe is AcircP1:

5'-CCCGCCGAAGCAACAAGGTACG.

88. The method according to claim 2 wherein the fungi are selected from the group consisting of *Aspergillus sydowii*, the forward primer is AsydoF1-1: 5'-CAACCTCCCACCCGaGAA, the reverse primer is versR1: 5'-CCATTGTTGAAAGTTTGGACTGATTTTA, and the probe is versP1: 5'-AGACTGCATCACTCTCAGGCATGAAGTTCAG.

89. The method according to claim 2 wherein the fungi are selected from the group consisting of *Aspergillus unguis*, the forward primer is AunguF1: 5'-CAACCTCCCACCCCTGAATACT, the reverse primer is AunguR1: 5'-TCACTCTCAGGCATGAAGTTCAG, and the probe is AcaesP1: 5'-CACTGTTGCTTCGGCGAGGAGCC.

90. The method according to claim 2 wherein the fungi are selected from the group consisting of *Aspergillus wentii*, the forward primer is AwentF1: 5'-CATTAACGAGTGGGGACCTAACC, the reverse primer is AauriR1: 5'-CGGGGGCCACGAAT, and the probe is AcircP1: 5'-CCCGCCGAAGCAACAAGGTACG.

91. The method according to claim 2 wherein the fungi

are selected from the group consisting of *Aspergillus niger*, the forward primer is AnigF1:

5'-GTCAGAGGCTATAACACACAGCAG, and the probe is CalbP1:

5'-TTTACCGGGGCCAGCATCGGTTT.

92. The method according to claim 2 wherein the fungi are selected from the group consisting of *Candida dubliniensis*, the forward primer is CdubF1: 5'-AGATCAGACTTGCTATTTTGCAAGTTA, the reverse primer is CdubR1: 5'-TAGGCTGGCAGTATCGTCAGA, and the probe is CdubP1: 5'-TTTACCGGGGCCAGCATCGGTTT.

93. The method according to claim 2 wherein the fungi are selected from the group consisting of *Candida (Pichia) guilliermondii*, the forward primer is CguiF1: 5'-CCTTCGTGSCGGGGTG, the reverse primer is CguiR1: 5'-GCAGGCAGCATCAACGC, and the probe is CguiP1: 5'-CCGCAGCTTATCGGGCCAGC.

94. The method according to claim 2 wherein the fungi are selected from the group consisting of *Candida haemulonii*, the forward primer is ChaeF1: 5'-GGAGCGACAACGAGCAGTC, the reverse primer is ChaeR1: 5'-AGGAGCCAGAAAGCAAGACG, and the probe is ChaeP1: 5'-ATGTAGTACAGCCCTCTGGGCTGTCCA.

95. The method according to claim 2 wherein the fungi are selected from the group consisting of *Candida haemulonii* type II, the forward primer is Cha2F1: 5'-ATCGGGTGGAGCGGAACT, the reverse primer is Cha2R1: 5'-CGAAGCAGGAACCATCTGAGA, and

the probe is Cha2P1: 5'-AAGTGGGAGCTGATGTAGCAACCCCC.

96. The method according to claim 2 wherein the fungi are selected from the group consisting of *Candida krusei*, the forward primer is CkruF1: 5'-CTCAGATTTGAAATCGTGCTTTG, the reverse primer is CkruR1: 5'-GGGGCTCTCACCCCTCCTG, and the probe is CkruP1: 5'-CACGAGTTGTAGATTGCAGGTTGGAGTCTG.

97. The method according to claim 2 wherein the fungi are selected from the group consisting of *Candida lipolytica*, the forward primer is ClipF1: 5'-TAGCGAGACGAGGGTTACAAATG, the reverse primer is ClipR1: 5'-CGTCGGTGGCAGTGTGGA, and the probe is ClipP1: 5'-CCTTCGGGCGTTCTCCCCTAACC.

98. The method according to claim 2 wherein the fungi are selected from the group consisting of *Candida lusitanae*, the forward primer is ClusF1: 5'-GGGCCAGCGTCAAATAAAC, the reverse primer is ClusR1: 5'-CGCAGGCCTCAAACAAACA, and the probe is ClusP1: 5'-AGAATGTGGGCGTGCCTTCG.

99. The method according to claim 2 wherein the fungi are selected from the group consisting of *Candida maltosa*, the forward primer is CmalF1: 5'-GGCCAGCATCAGTTTGGAC,

101. The method according to claim 2 wherein the fungi are selected from the group consisting of *Candida parapsilosis*, the forward pPrimer is CparF1: 5'-GATCAGACTTGGTATTTTGTATGTTACTCTC, the reverse primer is CparR1: 5'-CAGAGCCACATTTCTTTGCAC, and the probe is CparP1: 5'-CCTCTACAGTTTACCGGGCCAGCATCA.

101. The method according to claim 2 wherein the fungi are selected from the group consisting of *Candida sojae*, the forward primer is CsojF1: 5'-CGGTTGTGTGTTATAGCCTTCGTA, the reverse primer is CsojR1: 5'-ATCATTATGCCAACATCCTAGGTAAT, and the probe is CtropP2: 5'-CGCAGTCCTCAGTCTAGGCTGGCAG.

102. The method according to claim 2 wherein the fungi are selected from the group consisting of *Candida tropicalis*, the forward primer is CtropF1: 5'-GCGGTAGGAGAATTGCGTT, the reverse primer is CtropR2: 5'-TCATTATGCCAACATCCTAGGTTTA, and the probe is CtropP2: 5'-CGCAGTCCTCAGTCTAGGCTGGCAG.

103. The method according to claim 2 wherein the fungi are selected from the group consisting of *Candida viswanathii*, the forward primer is CvisF1: 5'-CGGCGGAGCAATCGCGT, the reverse primer is CvisR1: 5'-TCTAGGCTGGCAGTATCCACG, and the probe is CvisP1: 5'-AATGTGGCAGCGGCTCGGC.





5'-GAAAGTTTTAAATAATTTATATTTTCACTCAGAgTA, and the probe is PenP2: 5'-CGCGCCCGCCGAAGACA.

108. The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium atramentosum*, the forward primer is PgrisF1-1: 5'-ACCTGCGGAAGGATCATTtCT, the reverse primer is PatraR1: 5'-CCCCGGCGGCCATA, the probe is PenP3: 5'-TCCAACCTCCCACCCGTGTTTATTT.

109. The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium aurantiogriseum*, the forward primer is PauraF3: 5'-CGCCGGGGGGGCTTC, the reverse primer is PauraR1-1: 5'-GAAAGTTTTAAATAATTTATATTTTCACTCAGAgTT, the probe is PenP2: 5'-CGCGCCCGCCGAAGACA.

110. The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium aurantiogriseum/polonicum/viridicatum/freii*, the forward primer is PexpaF1-1: 5'-TTACCGAGTGAGGGCCgTT, the reverse primer is PauraR6: 5'-CCCGGCGGCCAGTA, the probe is PenP3: 5'-TCCAACCTCCCACCCGTGTTTATTT.

111. The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium*

*canescens*, the forward primer is PcaneF1:

5'-TTACCGAGCGAGAATTCTCTGA, the reverse primer is PcaneR1:

5'-AGACTGCAATTTTCATACAGAGTTCA, the probe is

PsimpP1: 5'-CCGCCCTCAAGGCCGCC.

112. The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium citreonigrum*, the forward primer is PcteoF1-1:

5'-TGTTGGGCTCCGTCCTCtTC, the reverse primer is PcteoR1-1:

5'-CGGCCGGGCTtCAG, the probe is PenP7: 5'-CCGAAAGGCAGCGGCCGC.

113. The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium coprophilum* the forward primer is PcoprF1-1:

5'-GGGTCCAACTCCCACTCA, the reverse primer is PchryR1-1:

5'-GAAAGTTTAAATAATTTATATTTTCACTCAGAgTA, the probe is PenP1:

5'-CGCCTTAACTGGCCGCGG.

114. The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium crustosum*, the forward primer is PcrusF1: 5'-CGCCGGGGGGGCTTA, the reverse primer is PauraR1-1:

5'-GAAAGTTTAAATAATTTATATTTTCACTCAGAgTT,

115. The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium digitatum*, the forward primer is PaethF1-1: 5'-CGGGGGGCTCtCGCT, the reverse primer is PdigiR1: 5'-CGTTGTTGAAAGTTTAAATAATTTcGT, the probe is PenP2: 5'-CGCGCCCGCCGAAGACA.

116. The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium expansum*, the forward primer is PexpaF2-1: 5'-TCCCACCCGTGTTTATTTACaTc, the reverse primer is PexpaR1: 5'-TCACTCAGACGACAATCTTCAGG or PexpaR1-1: 5'-TCACTCAGACGACAATCTTctGG, the probe is PenP1: 5'-CGCCTTAACTGGCCGCCGG.

117. The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium frezii*, the forward primer is PfreiF1: 5'-TCACGCCCCCGGGT, the reverse primer is PauraR1-1: 5'-GAAAGTTTTAAATAATTTATATTTTCACTCAGAgTT, the probe is PenP2: 5'-CGCGCCCGCCGAAGACA.

118. The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium glandicola*, the forward primer is PglanF1-1: 5'-CCGGGGGGCTTtCGT, the reverse primer is PchryR1:

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5'-GAAAGTTTTAAATAATTTATATTTTCACTCAGACTA, the probe is  
PenP2: 5'-CGGCGCCCGCCGAAGACA.

119. The method according to claim 2 wherein the  
fungi are selected from the group consisting of *Penicillium*  
*griseofulvum*, the forward primer is PgrisF1-1:

5'-ACCTGCGGAAGGATCATTtCT, the reverse primer is PchryR6:

5'-CCCGGGCGGCCAGTT, the probe is PenP3:

5'-TCCAAACCTCCCACCCGTGTTTATTT.

120. The method according to claim 2 wherein the  
fungi are selected from the group consisting of *Penicillium*  
*hirsutum*\*, the forward primer is PhirsF1-1: 5'-GCCGGGGGGCTCAtA,  
the reverse primer is PauraR1-1:

5'-GAAAGTTTTAAATAATTTATATTTTCACTCAGAgTT, the probe is

PenP2: 5'-CGCGCCCGCCGAAGACA.

121. The method according to claim 2 wherein the  
fungi are selected from the group consisting of *Penicillium*  
*implicatum*, the forward primer is PimplF1: 5'-GCCGAAGACCCCCCTGT,  
the reverse primer is PimplR1: 5'CGTTGTTGAAAGTTTGAAGTATTGT,  
the probe is PimplP1: 5'-AACGCTGTCTGAAGCTTGCAGTCTGAGC.

122. The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium* *marneffei*, the forward primer is PmarF1: 5'-GAAAGTTTTAAATAATTTATATTTTCACTCAGACTA, the reverse primer is PmarR1: 5'-CGGCGCCCGCCGAAGACA, the probe is PenP2: 5'-CGGCGCCCGCCGAAGACA.

the reverse primer is PislalR1: 5'-GGCAACGCGGTAACGGTAG,  
the probe is PislalP1: 5'-AGCCCAACCTCCCAACCCGTG.

123. The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium italicum*, the forward primer is PitalF1-1:  
5'-CTCCCACCCGTGTATTATTTAtCA, the reverse primer is PexpaR1:  
5'-TCACTCAGACGACAATCTTCAGG or PexpaR1-1:  
5'-TCACTCAGACGACAATCTTctGG, the probe is PenP1: (+)  
5'-CGCCTTAACTGGCCGCGCGG.

124. The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium melinii*, the forward primer is PmeliF1-1:  
5'-CAGGGCTTGTGTGTGGtCT, the reverse primer is PmeliR1:  
5'-GGGCCTACAAGAGCGGAA, the probe is PenP7: 5'-  
CCGAAAGGCAGCGGCGGC.

125. The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium miczynskii*, the forward primer is PmiczF1-1:  
5'-GTGTTTAACGAACCTIGTTGCaTT, the reverse primer is PmiczR1-1:  
5'-CTCAGACTGCATACTTCAGACaGA, the probe is PsimpP1:  
5'-CCGCCTCAGCGCGCGCC.

127. The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium purpurogenum*, the forward primer is PpurpF1:  
5'-AGGATCATTACTGAGTGCGGA, the reverse primer is PpurpR1:  
5'-GCCAAAGCAACAGGGTATTC, the probe is PpurpP1: 5'-  
CCCTCGCGGGTCCAACCTCC.

128. The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium raistrickii*, the forward primer is PgrisF1-1:  
5'-ACCTGCGGAAGGATCATTtCT, the reverse primer is PraisR1:  
5'-CCCGGCGGOCAGAC, the probe is PenP3:  
5'-TCCAAACCTCCCAACCGTGTtTATTt.

129. The method according to claim2 wherein the fungi are selected from the group consisting of *Penicillium restrictum*,

130. The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium sclerotiorum*, the forward primer is P sclrF1:

5'-TTCCCCCGGGGAACAGG, the reverse primer is P sclrR1:

5'-GCCCCATACGCTCGAGGAT, the probe is P sclrP1: 5'-  
CCGAAAGGCACTGGCGGCAC.

131. The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium simplicissimum/ochrochloron*, the forward primer is PsimpF2-1:

5'-CGCCGAAGACACCATTCATCT, the reverse primer is PsimpR4-1:

5'-CTCAATTCTGCAATTCACATAACG, the probe is PsimpP2:

5'-TGTCTGAAGATTGCAGTCTGAGCGATTAGC.

132. The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium variable*, the forward primer is PvarbF1: 5'-GCCGGGGGGCTTCT, the reverse primer is PvarbR1: 5'-TCTCACTCAGACTCACTGTTTCAGG, the probe is PvarbP1: 5'-AGGTTCTAGGGTGCTTCGGCGG.

133. The method according to claim 2 wherein the fungi are selected from the group consisting of *Penicillium verrucosum*\*, the forward primer is PverrF2: 5'-CGGGCCCGCCTTTG, the reverse primer is PverrR1: 5'-GAAAGTTTAAATAATTTATATTTTCACTCAGACTT, the probe is PenP2: 5'-  
CGCGCCCGCCGAAGACA.



135. The method according to claim 2 wherein the fungi are selected from the group consisting of *Ulocladium* *atrum*, the forward primer is UatrmF2: 5'-CGGGCTGGCATCCTTC, the reverse primer is UatrmR2: 5'-CTGATTGCAATTACAAAAGGTTTATG, the probe is UloP1: 5'-TGAATTATTCACCCGTGTCTTTTGCGTACTTCT.

136. The method according to claim 2 wherein the fungi are selected from the group consisting of *Ulocladium chartarum*, the forward primer is UcharF1-1:  
5'-AGCGGGCTGGAATCCaTT, the reverse primer is UcharR1-1:  
5'-CTGATTGCAATTACAAAAGGTTgAAT, the probe is UlcP1:  
5'-TGAATTATTTCACCCCTGTCTTTTGCGTACTTCT.

137. The method according to claim 2 wherein the bacteria are selected from the group consisting of *Legionella* maceachernii, the forward primer is LmaceF1:

[illegible]

138. The method according to claim 2 wherein the bacteria are selected from the group consisting of *Legionella micdadei*, the forward primer is LmicdF1:

5'-GGTGGTTTATAAGTTATCTGTGAAATTC the reverse primer is

PmicdR1: 5'-CACTACCCCTCTCCTATACTCAAAGTCTC the probe is

LmicdP1: 5'-AGTCTTATCTGACCACCCTAGGTTGAGCCCA.

139. The method according to claim 2 wherein the bacteria are selected from the group consisting of *Legionella pneumophila* the forward primer is LpneuF1:

5'-CGGAATTACTGGGCGTAAAGG the reverse primer is PpneuR1:

5'-GAGTCAACCASTATATCTGACCGT the probe is LpneuP1: 5'-

AAGCCAGGAATTTACAGATAACTTAATCAACCA.

140. The method according to claim 2 wherein the bacteria are selected from the group consisting of *Legionella sainthelensi/cincinnatiensis*, the forward primer is LsainF1:

5'-CGTAGGAATATGCCTTGAAGACT the reverse primer is PsainR1:

5'-AAGGTCCCCAGCTTTTGT the probe is LsainP1: 5'-

AGACATCATCCGSTATTAGCTTGASTTTCCC.

FIG. 1

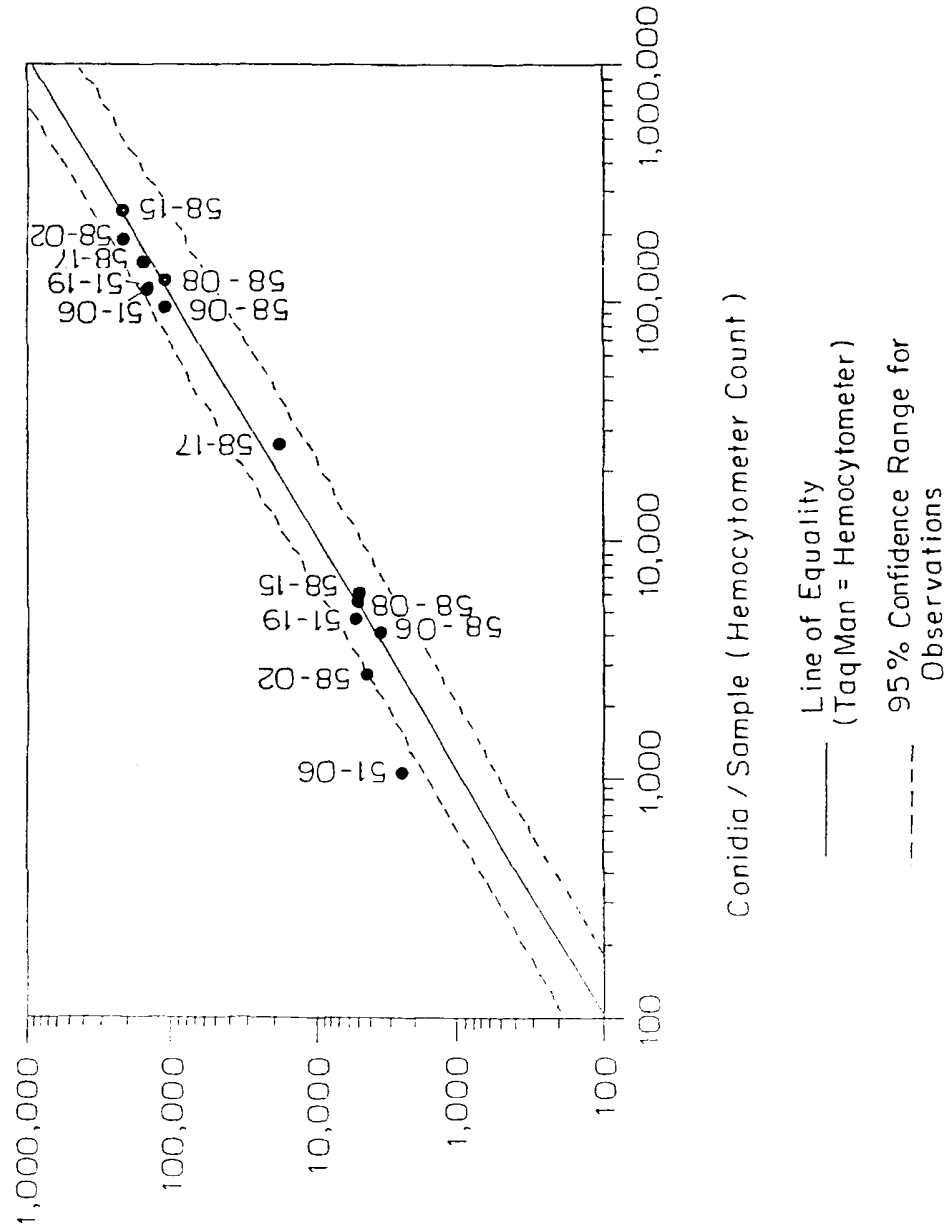
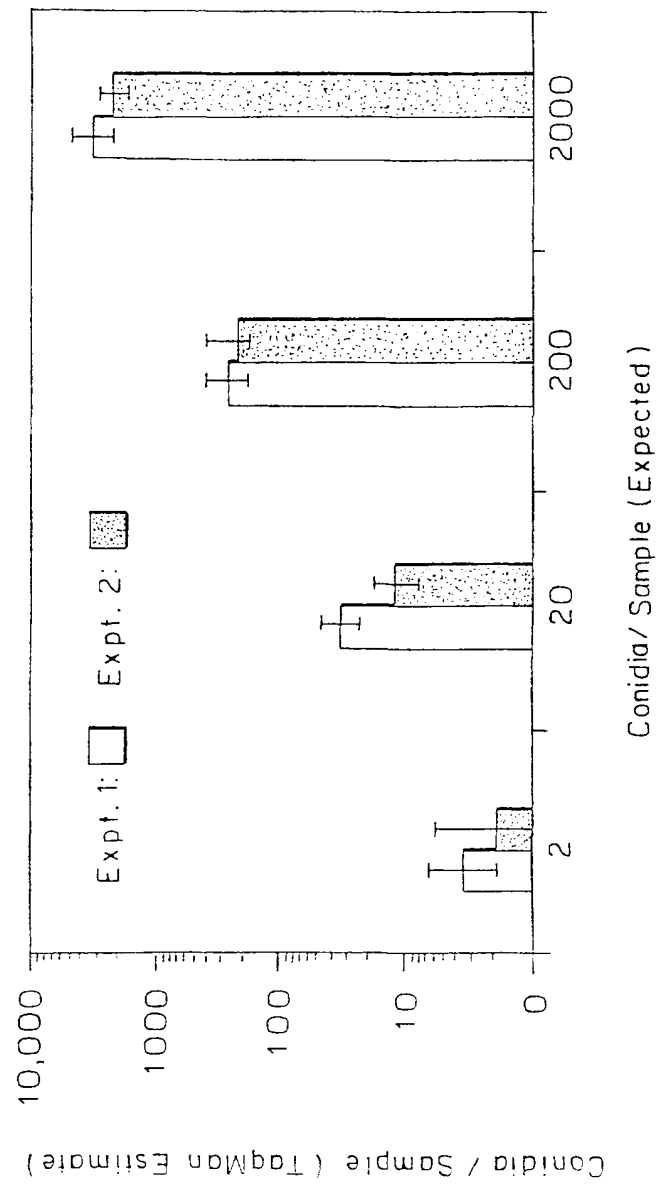
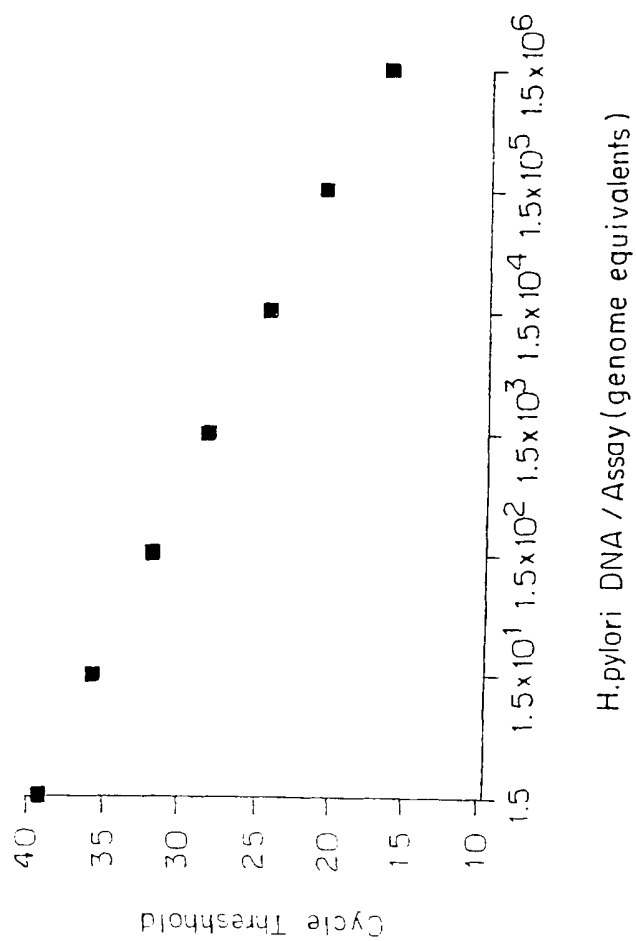


FIG. 2



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FIG. 3



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FIG. 4

